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Signature of Supervisor

25/08/20
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A STUDY ON FLAVONOIDS TO AMELIORATE THE CAUSE OF VARIOUS NEURODEGENERATIVE DISEASES.

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF TECHNOLOGY
IN
BIOMEDICAL ENGINEERING

Submitted by:

SHRUTI THAREJA

2K18/BME/03

Under the guidance of:

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AUGUST, 2020

CANDIDATE'S DECLARATION

I, Shruti Thareja (Roll No.: 2K18/BME/03), student of M.Tech Biomedical Engineering, hereby declare that the project dissertation titled " **A Study On Flavonoids To Ameliorate The Cause Of Various Neurodegenerative Diseases**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship, or other similar title or recognition.

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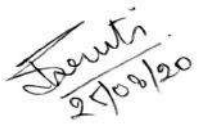

SHRUTI THAREJA

2K18/BME/03

M.TECH BIOMEDICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY, DELHI

CERTIFICATE

This is to certify that the M.Tech dissertation entitled **"A STUDY ON FLAVONOIDS TO AMELIORATE THE CAUSE OF VARIOUS NEURODEGENERATIVE DISEASES."** submitted by **Shruti Thareja (2K18/BME/03)** in partial fulfillment of the requirement for the award of the degree of Master of Technology from Delhi Technological University, is an authentic record of the candidate's own work carried out by her under my guidance. To the best of my knowledge this work has not been submitted in part and full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 25.08.2020

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ABSTRACT

Neurodegenerative diseases are still incurable and the current medications that are available can just control the symptoms of the diseases with a number of harmful side effects. In order to reduce these effects, natural compounds such as secondary metabolites can be thought of as a replacement. One of the categories of the secondary metabolites is the flavonoids that are known to possess a number of significant health benefits for the humans and thereby can as a replacement for the conventional drugs that are approved for the various NDDs because of their better effectiveness and fewer or no side effects. These flavonoids can be obtained from various natural sources such as the Plantae Kingdom. A part of the Plantae Kingdom is herbs and spices, which are used in food and beverages since time immemorial to enhance the color, flavor, and aroma. These herbs and spices are the easily available things in a household. For this, a database of the flavonoids that are present in the Indian herbs and spices was created, and they formed the basis of this study. The study deals with the screening of the flavonoids by predicting their ADME predictions, toxicity, bioactivity, and docking. And thereby docking the screened flavonoids with the drug targets of the various NDDs, in order to limit the utilization of the conventional drugs. By this *in silico* study, we would be able to predict the pharmacokinetic properties and compare the binding affinities of the conventional drugs with the screened flavonoids.

Keywords: Drug-Likeness, Bioactivity, Flavonoids, *in silico*, Indian herbs and spices, Molecular Docking, Neurodegenerative Diseases, Pharmacokinetic Properties.

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LIST OF SYMBOLS AND ABBREVIATIONS

-OH : Hydroxyl group

Aβ : Amyloid Beta

AChE : Acetylcholinesterase

AD : Alzheimer's Disease

ADL : Activities of Daily Living

ADMET : Absorption, Distribution, Metabolism, Excretion, Toxicology

ALS : Amyotrophic Lateral Sclerosis

APP : Amyloid Precursor Protein

APOE : Apolipoprotein E

ATP : Adenosine Triphosphate

BBB : Blood Brain Barrier

BDNF : Brain Derived Neutrophic Factor

CBD : Corticobasal Degeneration

ChEBI : Chemical Entities of Biological Interest

CNS : Central Nervous System

COMT : Catechol-O-Methyltransferase

COX : Cyclooxygenase

CYP : Cytochrome P450

DA : Dopamine

DBS : Deep Brain Stimulation

DEGs : Differentially Expressed Genes

DNA : Deoxyribonucleic Acid

EC : Entorhinal cortex

EGCG : Epigallocatechin gallate

FAD : Familial Alzheimer's Disease

FDA : U.S. Food and Drug Administration

GEO : Gene Expression Omnibus

GI : Gastrointestinal

GPI : Globus Pallidus Interna

GSK3β : Glycogen Synthase Kinase 3 Beta Pathway

HBA : Hydrogen Bond Acceptor

HBD : Hydrogen Bond Donor

HD : Huntington's Disease

IL : Interleukin

IUPAC : International Union of Pure and Applied Chemistry

L-dopa : Levodopa

MAO-B : Monoamine Oxidase B

MAPKs : Mitogen-Activated Protein Kinases

MSA : Multiple System Atrophy

NDDs : Neurodegenerative Diseases

NIH : National Institute of Health

NFT : Neurofibrillary Tangles

NFκB : Nuclear Factor kappa-light-chain-enhancer of Activated B Cells

NPH : Normal Pressure Hydrocephalus

P-gp : Permeability of Glucoprotein

PD : Parkinson's Disease

PDB : Protein DataBank

PI3K : Phosphoinositide 3-kinase

PNS : Peripheral Nervous System

PSEN1 : Presenilin 1

PSEN2 : Presenilin 2

PSP : Progressive Supranuclear Palsy

QC : Quercetin

RCSB : Research Collaboratory for Structural Bioinformatics

RO5 : Lipinski's Rule of Five

ROS : Reactive Oxygen Species

RNA : Ribonucleic Acid

SCN : Soluble Carrier Family 22 Member 6 (S22A6)

SDF : Spatial Data File

SMILES : Simplified Molecular Input Line Entry System

SN : Substantia Nigra

STN : Subthalamic Nucleus

STRING : Search Tool for the Retrieval of Interacting Genes/Proteins

VAT-1 : Synaptic Vesicular Membrane Transport

VP : Vascular Parkinsonism

TNF-α : Tumor Necrosis Factor Alpha

WHO : World Health Organization

1. INTRODUCTION

Nature remains to be the most important source of medicines for human welfare, since time illegitimate. The plant kingdom is a great source of bioactive compounds, which due to their intrinsic properties, can be used for treating various diseases in humans related to the cardiovascular system, immune system, and nervous system. The plants in recent times, have been the most common topic for scientific studies due to their medicinal properties and also because of the low toxicity, economic viability of the plants. Because of these properties, plants can be used as a good source of phytopharmaceuticals. And, there is a global need to explore the plant kingdom in order to improve mental health, cognitive functions, and also to enhance the focus and concentration.

Nature is a repository of biological and chemical diversity [1]. Since many decades, it has been seen that the escalation of an immense number of scientific studies that have been focused on the activity of the non-nutritional compounds present in the diet and are able to prevent the incidence of various degenerative diseases like cancer, cardiovascular diseases and neurodegenerative diseases [2]. Medicinal plants contain various phytochemicals that are extractable and can be utilized for various scientific surveys. Many secondary metabolites are used in the pharmaceutical industry. In recent times, medicinal plants have gained a lot of importance due to their lesser side effects in comparison to the synthetic drugs and also due to the fact that they can meet the medicinal needs of the increasing human population.

The later part of human life is usually affected by one or the other neurological condition like loss of cognitive ability, loss of memory, etc. A prolonged state of these symptoms leads to a condition called Dementia. Dementia is usually the first stage of any neurological disorder.

Neurodegenerative Disorder (Greek neuro – “nerval” and Latin degenerate, “to decline” or “to worsen”), is a heterogeneous group of progressively deteriorating conditions that act on specific areas of the CNS and the PNS, thereby heading to gradual and progressive cognitive impairments, depending upon the type of the nerve cell that is undergoing degeneration in that particular disease [1]. The major characteristic features of the neurodegenerative disorders are ataxias (impairment in movement) and dementia (decline in memory) [3]. Some neurodegenerative diseases can be due to genetic mutations, and some are also related to the surrounding hazardous environmental conditions. Along with brain aging, there can be many other causes of neurodegeneration also like: inflammation, oxidative stress, deposition of

aggregated proteins, activation of various apoptotic factors. However, till date, their main causes are still not known. The neurodegenerative disorders are:

1. Alzheimer's Disease (AD)
2. Parkinson's Disease (PD)
3. Huntington's Disease (HD)
4. Amyotrophic Lateral Sclerosis (ALS)
5. Multiple Sclerosis
6. Schizophrenia
7. Seizure disorders, etc.

Out of the above-mentioned disorders, the most common ones are Alzheimer's Disease and Parkinson's Disease. They are responsible primarily is affecting the life span and the life quality of the elderly [3].

Despite the progress in examining the pathology of the various neurodegenerative diseases, the cure is still unknown. Whatever drugs and treatments are known till date, they are just capable of controlling the symptoms and are unable to completely cure the root cause of the disease and make the patient disease-free. This is because the disease is detected very late as the symptoms appear later than the onset of disease, so it is difficult to analyze whether the person is suffering from the disease or not.

Herbs and spices have been used in a multiple number of ways since time immemorial. Since ancient times, herbs and spices are being added to the food for enhancement of flavor and also to improve the organoleptic properties [4]. As per the Food and Drug Administration (FDA), spices are: " aromatic vegetable substances, in the whole, broken, or the ground form, whose significant function is seasoning in food rather than nutrition" [5]. Spices have been used for purposes such as medicine, religious, cosmetics, vegetables or perfumery, other than flavoring [6]. Herbs and spices (especially in the dried form) contain a high content of polyphenols and other physiologically active phytochemicals [7].

Phytochemicals are a varied group of bioactive compounds that are present in the plants[8] and include flavonoids, alkaloids, terpenoids, phenols and lignans. Due to their wide range of chemical, biochemical, and molecular characteristics, they can be of great interest for neurodegenerative diseases[8]. Phytochemicals have proved to be promising candidates for various pathological conditions such as modulation of multiple signaling pathways and behaving as antioxidant and anti-inflammatory agents [9], agents against cancer and various

neurodegenerative diseases or in some cases as antifungal agents too [10]. Phytochemical rich diets are usually associated with increased longevity and a wide range of health benefits such as a decreased incidence of cardiovascular diseases and a slowed progression of cerebrovascular diseases [11]. The most common and abundant phytochemicals are the flavonoids.

Flavonoids are polyphenolic compounds. They are naturally occurring, biologically active compounds, that are abundantly found in fruits and vegetables [12]. Due to the variations in the C-ring in the structure of the flavonoids, they are classified as flavonols, flavones, isoflavones, flavanol, flavonones, and anthocyanins [13]. Flavonoids and their metabolites have great effects on human and animal health, because of their antioxidant properties. Hence due to these antioxidant properties, flavonoids become the potential candidates for the study on neurodegenerative diseases. Flavonoids are also important for, the suppression of lipid peroxidation, modulation of gene expression, inhibition of inflammatory inhibitors. Flavonoids also help to maintain the endogenous antioxidant status of the neurons and thereby protecting them from any kind of degeneration [8]. They are the only class of secondary metabolites that can cross the blood brain barrier (BBB). Hence, their multiple effects have drawn the interest of the scientists towards flavonoids and discovering their role in neurodegenerative diseases.

The main objectives of this project are:

1. To create a database of the Indian spices and herbs along with their constituent flavonoids.
2. To find out the 3-D structure of the proteins, flavonoids and conventional drugs.
3. To estimate the molecular and drug-likeness properties of the flavonoids.
4. To estimate the pharmacokinetic properties of the flavonoids.
5. To predict the toxicity of the screened flavonoids.
6. To predict the binding affinity between the proteins and the flavonoids and the conventional drugs and compare them.
7. To predict their bioactivity and antioxidant activity of the screened flavonoids.

2. REVIEW OF LITERATURE

2.1 NEURODEGENERATIVE DISEASES: AN OVERVIEW

The ongoing industrialization, changes in life style, and excessive use of herbicides and pesticides and other toxic chemicals that are used in the production of food materials are seriously threatening to the life of humans and are posing various health hazards. These toxic chemicals produce neurotoxins that affect the chemical transmission among the neurons and thereby causing neurodegenerative diseases [14].

Millions are affected yearly with neurodegenerative diseases worldwide. According to the World Health Organization (WHO), 737 million people were affected with neurodegenerative diseases worldwide in the year 2009, all of these people were of 60 years of age or more. This no is expected to increase to 2 Billion by the year of 2050.

Neurodegenerative diseases are a group of progressively deteriorating conditions that affect certain areas of the CNS and the PNS, leading to a gradual decrease in cognitive and movement impairments depending upon the type of the nerve cell that is undergoing degeneration [1]. There are various causes of these neurodegenerative diseases such as protein degradation [15], various environmental factors [16], familial history [18,19], mitochondrial defects [19], abnormal protein accumulation in neurons [20], etc. however, the most general cause of these diseases is aging [19].

There are many Neurodegenerative Diseases, some of them have been mentioned below:

- Alzheimer's Disease (AD)
- Parkinson's Disease (PD)
- Huntington's Disease (HD)
- Amyotrophic Lateral Sclerosis (ALS)
- Multiple Sclerosis
- Schizophrenia
- Seizure disorders, etc.

Alzheimer's Disease and Parkinson's Disease are the most common NDDs. They occur in 40-60% of the patients [21].

The brain and spinal cord are made up of innumerable different neurons that have different functions, such as controlling movements, making decisions and providing sensory information

[1]. Since these neuronal cells cannot regenerate themselves, hence their degradation can be devastating. NDDs are thereby characterized by the gradual loss of the sensory and the motor neurons and also the loss of the ability of the mind to send sensory information to an external object [22]. Neurodegenerative diseases are divided into two different groups [1], namely:

1. Movement related problems, such as ataxia
2. Dementia and memory-related problems.

The biological mechanisms that are associated with neurodegenerative diseases are aggregation of proteins in neurons, oxidative stress, abnormal ubiquitination, mitochondrial dysfunction, depletion or inadequate synthesis of neurotransmitters, excitotoxicity of neurons as well as disarrangement, degradation of neurotransmitters in the synaptic cleft due to the increased activity of enzymes or blood brain barrier (BBB) [23].

The most common form of the NDDs is Dementia that is affecting millions worldwide and continues to affect them as the age progresses. It is not a disease; rather, it is a group of symptoms, leading to various other diseases or conditions [1], being characterized by chronic progressive mental disorder, affecting the memory, comprehension, thinking, language and calculation [1].

2.1.1 Developmental Stages of Neurodegenerative Diseases

There are three developmental stages of neurodegenerative diseases. In this section, each stage has been described briefly along with the symptoms that appear at each stage.

- **Retrogenesis:** The beginning of the NDDs is the malfunctioning of the cholinergic system of the basal forebrain, which reaches the entorhinal cortex and hippocampus that are responsible for the short and long-term memory [24]. This results in the modifications in the brain, which usually start 10-20 years before and the first signs of NDD are forgetfulness and short term memory loss [25]. The disease, with its further progression, starts affecting the cerebral cortex. This stage is linked with the clinical diagnosis of NDDs in patients, which include losing decision power, confusing in similar places, mood and personality changes, missing valuable things, increased anxiety, loss of spontaneity and sense of initiatives [24].
- **Cognitive Dysfunction:** There is a relation between neurodegeneration and toxic proteins. It is accompanied by the increase in pathological neurofibrillary plaques and tangles in the entorhinal cortex (EC), caudate, substantia nigra [24]. In order to keep

the memory alive, the connection between EC and hippocampus has to be maintained, and any difficulty in these two regions can disrupt the circuit and lead towards memory damage and disorder.

- **Gait Abnormality:** Predicting a gait abnormality indicates a disturbance in the cognitive functions. A term has been proposed, " Last-In-First-Out", which refers to the phenomenon in which the neural circuits that mature late in the developmental life cycle are more vulnerable to damage, i.e., neurodegeneration [26]. Disturbances in cognitive function are directly linked with higher gait disturbances and is one of the major symptoms of brain syndrome [27].

2.2 ALZHEIMER'S DISEASE

Alzheimer's Disease being the most common form of dementia and hence the neurodegenerative disorder which occurs among older people above the age of 60 years. Once, this disease was considered as a rare disease and now it has become the most common in almost every household and is affecting millions of people worldwide. It was discovered in 1906 by a German neuropathologist and psychiatrist named Alois Alzheimer and hence the disease is named after him.

AD is a type of dementia causing problems related to memory, thinking and behavior. Symptoms usually develop steadily and worsen over time, becoming severe enough that it starts interfering with day-to-day tasks. AD accounts for 60 to 80% of the total cases of dementia worldwide. The greatest known risk factor of AD is age, and the majority of people with this disease are usually 65 years or older. AD gets worsen with time and age, since it is a progressive disorder. In the initial stages, patients just have mild memory loss, but with gradual progression, they start losing the ability to carry on a conversation and response to the environment.

2.2.1 Effects of Alzheimer's Disease on the Brain

There are complex changes that occur in the brain during the onset and the progression of the disease. The changes in the brain start occurring a decade before the onset of the actual cognitive symptoms. The early stages of the AD are usually symptom-free, but there are toxic changes that are occurring in the brain. In AD, there are abnormal depositions of proteins form amyloid (Aβ) plaques and tau tangles (neurofibrillary tangles, NFT). Once, the health neurons stop functioning, they lose connections with the other neurons and therefore, they die. The

damage initially appears in the hippocampus and the entorhinal complex (EC) of the brain, which are primarily responsible for the memories. As the disease progresses, more and more neurons die, affecting the specific brain area to shrink. By the time, the disease has reached the end of the first stage, the tissue of the brain has been reduced to a greater extent. The difference between a healthy and AD affected brain has been shown below in figure 1.

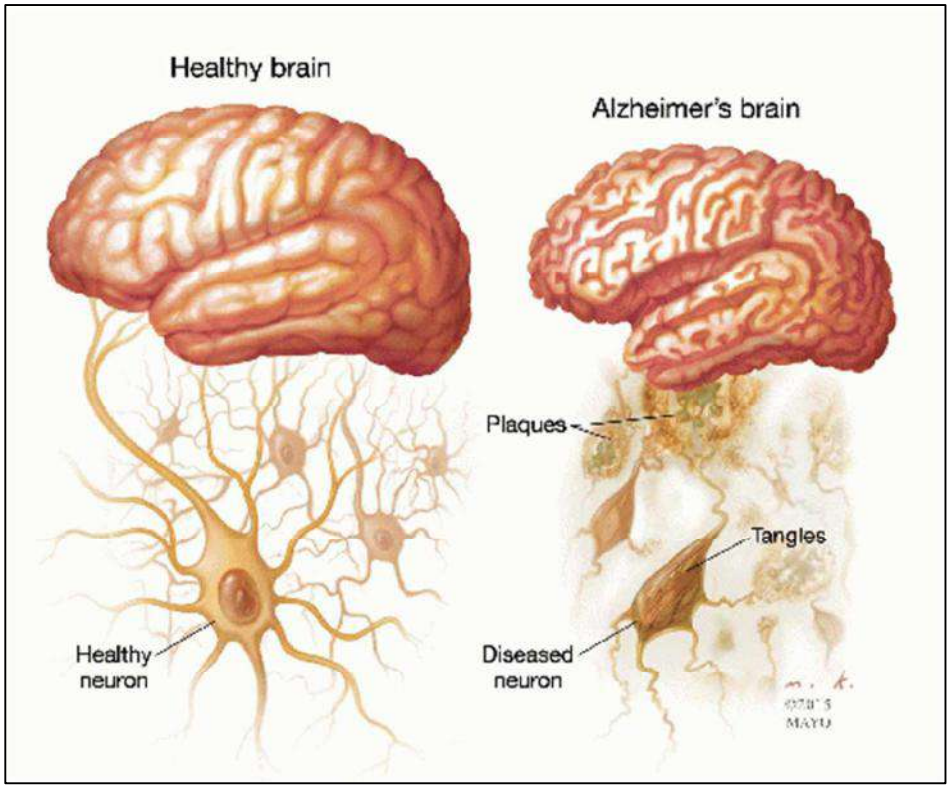

Figure 1: Difference between healthy brain and Alzheimer's brain. (Source: Mayo Clinic)

2.2.2 Causes of Alzheimer's Disease

Scientists feel that in people with early onset of AD, genetic mutations are usually the cause of the disease. And in those people with late-onset of AD, there can be a complex series of brain changes that can occur over time. These changes can be attributed to environmental, genetic, and lifestyle factors. Some of the main causes of AD have been described below:

- **Age-related changes in the brain:** As we all know that AD is an age-related disease that affects the people of age 65 and above. The main reason that it strikes in the later stage of life is that, as the age of the person progresses, the brain also undergoes certain changes. These changes are atrophy (shrinking) of certain parts of the brain, inflammation, production of free radicals (unstable molecules) and mitochondrial dysfunction (breakdown of energy production within the cell).

- **Genetics:** Another cause for AD is genetics. Due to genetics, one can say whether the disease was an early onset or late-onset. A gene called apolipoprotein E (APOE) is involved in the late-onset of AD. This gene has got several forms. One of them is the APOE ε4, which increases the person's risk of developing the disease and is also associated with the development of early-onset of AD. However, carrying the gene APOE ε4 does not necessarily mean that the person will develop the disease, as people who didn't carry this gene had developed AD according to the research.

  Also, a number of regions in the genome have been identified by the researchers that might be responsible for causing the disease to varying degrees.

  Early-onset AD is generally caused in people between the age group of 30 to 60, and less than 5 % of the people develop the disease. Most of the cases are due to the changes in one of the three genes for AD, and this type of disease is known as the early-onset familial Alzheimer's Disease or FAD.

  Most of the people with Down's Syndrome can develop AD. This is because, in the case of Down's Syndrome, there is an extra copy of chromosome 21, which is supposed to have the gene responsible for producing harmful amyloid.
- **Health, Environment and Lifestyle Factors:** Researchers suggest that beyond genetics, health, environment and lifestyle factors also play an important role in causing AD. A nutritious diet, physical activity, social engagement, and mentally stimulating pursuits have all been important in helping people to stay healthy as they age. These factors might also help to reduce the risk of cognitive decline and AD.

2.2.3 Types of Alzheimer's Disease

There are two types of AD:

- **Early-onset AD:** This type of AD is very rare; only 10% of the population have this type of AD. It usually occurs among the people between the age group of 30 and 60. Some cases are due to an inherited change in one of the three genes.

  The three single-gene mutations that are associated with the early-onset of AD are:
  - Amyloid Precursor Protein (APP) on chromosome 21
  - Presenilin 1 (PSEN1) on chromosome 14
  - Presenilin 2 (PSEN2) on chromosome 1

Gene mutations result in the production of abnormal proteins that are associated with the disease and play an important role in breaking down the APP. This breakdown leads to the generation of Amyloid Plaques, a hallmark of AD.

- **Late-onset AD:** Maximum cases of AD are of this type. People with the late-onset of AD start developing symptoms somewhere around the age of 60. Till date, no specific gene responsible for causing AD has been identified. But the presence of apolipoprotein E (APOE) on chromosome 19 does increase the risk of AD. The APOE is responsible for carrying out cholesterol and other types of fats in the bloodstream.

  There are three different forms of APOE. They are:

  - APOE ε2 is quite rare and might also help in providing protection against the disease. If AD occurs in some person having this allele, it would occur too late in the person.
  - APOE ε3 is the most common allele. It is believed to play a neutral role, i.e., neither decreasing nor increasing the risk of the disease.
  - APOE ε4 increases the risk of AD and is also associated with the early-onset of AD. Having one or two alleles of APOE ε4, increases the risk of the development of the disease. About 25% of the people carry one allele, and almost 2 to 3% of people carry two alleles. This gene increases the risk of development of the disease in the person. But it is not necessary that if the person carries the allele, he/she will develop a disease. In some cases, it has been seen that people who didn't carry the allele also developed the disease and those carrying the allele didn't develop the disease.

2.2.4 Signs and Symptoms of Alzheimer's Disease

An inability to retain the very recent events is the first symptom of AD. With the progression in the disease, impairment in different areas of cognition (for instance, language, abstract reasoning, and executive function or decision making), start occurring to unpredictable degrees and it archetypally coincides with difficulty occurring at work or in any social condition or various household activities [28]. Mood changes also accompany deteriorating memory[29]. Delusions and psychotic behavior are not naturally triggering signs but can occur at any time during the course of the disease[30]. The occurrence of psychosis during the initial stages of dementia suggests some other diagnosis like presence of dementia with Lewy Bodies [28].

A family history of dementia is considered to be one of the most important risk factors for AD [31]. About half of such cases result due to the mutations in the genes encoding APP, presenilin 1 or presenilin 2 [32]. The disease is also more concordant among monozygotic twins as compared to the dizygotic twins [33].

As the disease progresses, the conditions start getting worse and the following symptoms start appearing:

- Disorientation, confusion, and getting lost in familiar places
- Difficulty in planning or making decisions
- Problems with speech and language
- Personality changes, such as becoming aggressive, demanding and suspicious of others
- Problems in moving around without any assistance or performing self-care tasks
- Hallucinations or delusions
- Low mood or anxiety

2.2.5 Stages of Alzheimer's Disease

There are basically four stages of the AD. The disease along with their characteristics, has been discussed below in the following table:

Table 1: Changes that occur at various stages during progression of AD.

	Mild	Moderate	Severe
Activities of daily living (ADL)	Problems with the routine tasks	Needs help with basic ADL (e.g., feeding, dressing, bathing)	Progresses to total dependence on the caregiver
Behavior	Changes in personality and mathematical changes	Anxiety, suspicion, pacing, insomnia, agitation, wandering	Crying, screaming, groaning
Cognition	Confusions and memory loss, e.g. misplacing objects, forgetting names, disorientation	Difficulty in recognizing family and friends and chronic loss of memory	Loss of speech and misidentifies or is unable to recognize familiar people

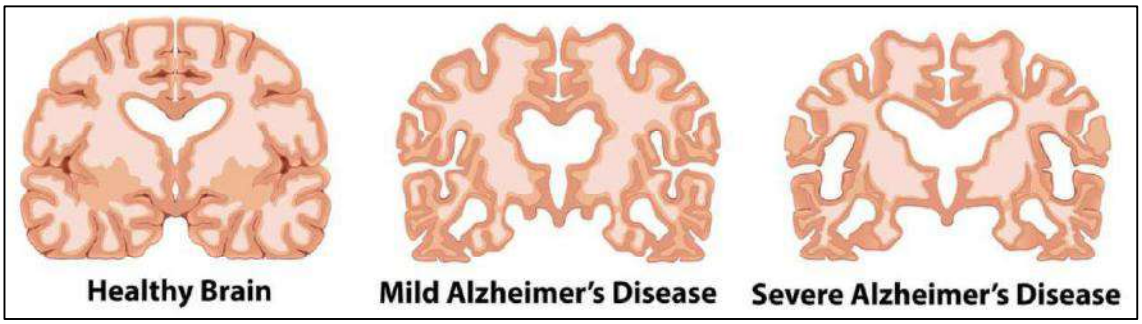

Figure 2: Progression of the Alzheimer's Disease (Source: National Institute on Aging)

2.2.6 Treatment of Alzheimer's Disease

A number of medicines have been prescribed for the treatment of AD. The various medicines are:

- Acetylcholinesterase (AChE) inhibitors

  AChE is an enzyme participating in the cholinergic neurotransmission. It breaks down acetylcholine into acetate ion and choline, which terminates the neurotransmission process [34]. The role of AChE inhibitors is to enhance the level of acetylcholine in the brain [1]. Hence AChE inhibitors play an important role by inhibiting the AChE from breaking down the acetylcholine and thereby continuing the neurotransmission process. Donepezil, galantamine, and rivastigmine are the medicines that are usually prescribed by the doctors for the people with early to mild AD. People with severe AD are also advised to take these medications, but in many cases, it has not proved to be effective. There are various side effects of these drugs, like nausea, loss of appetite, and vomiting.
- Memantine

  This medicine is not an AChE inhibitor, it works by blocking the effect of an excessive amount of the chemical called glutamate in the brain.

  Memantine is used for moderate or severe AD. It is usually prescribed for those patients that cannot take or are unable to tolerate the AChE inhibitors.

  It can be taken along with the AChE inhibitors also. It can cause certain side effects too. Side effects include headaches, dizziness and constipation, but these are usually temporary.

2.3 PARKINSON'S DISEASE (PD)

Globally, the second most common NDD is Parkinson's Disease. More than 10 million people are affected by PD worldwide, and it accounts for 2% of the population, older than 65 years of age. It was first described as a neurological syndrome by James Parkinson in the year 1817, hence the name of the disease.

PD is caused by the deficit of the dopaminergic neurons in the substantia nigra (SN) and striatum parts of the brain which further leads to a decrease in the dopamine (DA) level [35]. The loss of DA level to the striatum and the SN leads to an imbalance with neurotransmitters such as AChE and DA, resulting in PD symptoms [36]. The symptoms of the disease, gradually worsen over time and age since it is a progressive disorder. It leads to difficulty in walking and talking as the disease progresses. Both men and women can be affected by PD, but 50% more men are affected than by women.

2.3.1 Effect of Parkinson's Disease on the Brain

The property that makes it different from other movement disorders is that cell loss occurs only in a specific region of the brain, i.e., the SN. The nerve cells in this region appear dark or black when seen under the microscope, as the Latin for SN is a "black substance".

The neurons in the SN produce a neurotransmitter called dopamine. Dopamine helps in the regulation of movement. Thereby categorizing it as a movement disorder. In addition to the loss of dopamine, the protein α-synuclein is also affected in the disease. This protein tends to form aggregates that are called Lewy Bodies in the patients that are affected with PD.

As the disease progresses, more and more neurons die in the SN region of the brain, as can be seen in figure 3. There is a very small proportion of the people getting affected by the disease, which is an early onset of the disease. The early onset of the disease usually starts at the age of 40 years in some cases and in some can start after 50 years of age.

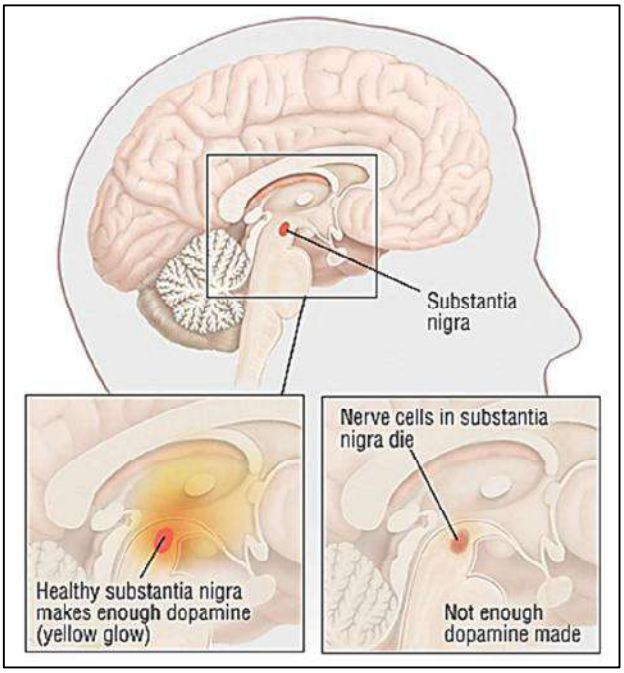

Figure 3: Difference between healthy brain and Parkinson's Disease brain. (Source: Harvard Medical School)

2.3.2 Causes of Parkinson's Disease

The cause of Parkinson's Disease is still unknown. It can be possible that there can be more than one cause of the disease. Scientists believe that both environmental factors and genetics play an important role in causing the disease. Some of the factors have been described below:

- **Genetics:** A number of genetic factors have shown to increase the risk of the disease in a person. The pathway of how genetic factors increase the susceptibility of the disease are still unknown. It can run in families, being passed on from one generation to another, but such cases are rare.

  The most common genetic effect that triggers the disease in a person is the mutation in the gene LRRK2. Mutations in the protein α-synuclein are also known to trigger the disease.
- **Environmental Factors:** Certain environmental factors such as exposure to pesticides or herbicides that are used in farming and traffic or industrial pollution may trigger the condition. Traumatic brain injury, known to alter the levels of consciousness, also increases the risk of the disease. Exposure to heavy metals can be thought of as a contributor to the onset of the disease.
- **Other causes:** "Parkinsonism" is the term that is used to describe the symptoms like tremors, muscle rigidity, and slowness of movement. Parkinson's Disease is the most

common type of parkinsonism. There can be multiple causes included under parkinsonism such as antipsychotic medications that can affect the brain.

2.3.3 Types of Parkinson's Disease

There are following types of Parkinson's Disease:

- **Primary Parkinsonism or Idiopathic PD:** It is the most common type of PD. The term idiopathic signifies that the cause is unknown. The most common symptoms of this are tremor, rigidity, and slowness of movement.
- **Secondary Parkinsonism:** It is also called Parkinsonism Syndrome or Atypical Parkinsonism or Parkinson's Plus. In this condition, the cause of the disease is known, but it is difficult to differentiate it from the primary parkinsonism except for the fact that it doesn't respond to the dopaminergic medications like levodopa. Secondary parkinsonism is of the following types:
    - **Multiple System Atrophy (MSA):** This is the advanced form of the idiopathic PD. In this, there is an overproduction of the protein α-synuclein. This results in damaging major parts of the brain. It progresses at a faster rate than the idiopathic PD. Its average onset is in the mid- '50's. It is difficult to distinguish it from normal PD, other than the fact that it doesn't respond to the PD medications. Its symptoms include ataxia and dysfunction in the automatic nervous system.
    - **Drug-induced Parkinsonism:** This condition is caused as a result of the side effects of the medications, especially those affecting brain dopamine levels. This is the most common form of secondary parkinsonism. It is difficult to differentiate it from the idiopathic PD.
    - **Vascular Parkinsonism (VP):** It can also be considered as a cerebrovascular disease. This type of parkinsonism is caused by a series of small strokes that can cause the death of certain areas of the brain. VP usually affects the lower body and memory loss. VP doesn't respond to the normal PD medications. It becomes more common with age, especially with the people who are affected by diabetes.
    - **Normal Pressure Hydrocephalus (NPH):** This condition is similar to VP. This condition can be treated by removing the spinal fluid as a short-term treatment,

and for the long-term treatment, a lumbar puncture can be done to permanently divert the spinal fluid.

- **Corticobasal Degeneration (CBD):** It is the least common type of Parkinsonism. It is caused due to the build-up of the protein tau, which damages various parts of the brain. It usually starts by affecting limb on one side of the body and then slowly spread to other parts with time. It typically begins after the age of 60 and progresses more rapidly than the PD.
- **Progressive Supranuclear Palsy (PSP):** It is the most common type of secondary parkinsonism. The cause of PSP is similar to that of CBD, i.e., the build-up of the protein tau, leading to damage in various parts of the brain. Those with PSP often have problems in swallowing, difficulty in producing speech, memory loss and sleeping problems.

2.3.4 Signs and Symptoms of Parkinson's Disease

PD has the following main symptoms:

- Trembling in hands, leg, jaw, or head.
- Stiffness in the limbs and the trunk.
- Bradykinesia (slowness of movement)
- Rigidity (muscle stiffness)
- Impaired balance and coordination

Other symptoms may include depression, problems in swallowing, chewing, speaking problems, skin problems, sleep disturbances. The main symptom is memory loss. It is difficult to test the onset of the disease.

Symptoms and the rate of progression of the disease vary from person to person. The symptoms can be physical or cognitive.

Physical symptoms of PD include: balancing problems, anosmia (loss of smell), nerve pain, constipation, dizziness, blurred vision or fainting, hyperhidrosis (excess of sweating), insomnia, drooling.

Cognitive and Psychiatric symptoms include: depression, anxiety, dementia, which may include hallucinations in some people, memory problems, problems in decision making and organization.

People with PD usually develop a parkinsonian gait, which makes them lean forward and reduce swinging of arms. They may also have trouble in continuous movement. Symptoms usually occur on one side of the disease and then spread to the rest of the body.

2.3.5 Treatment of Parkinson's Disease

There is currently no treatment available for the disease. The treatment that is available is to control the symptoms of the disease and to lead a quality life. These treatments include:

- Supportive therapies
- Medications
- Surgery

Supportive therapies: It includes physical, occupational and speech therapies. Physical therapy can improve the gait developed due to the disease and can help in improving the right exercise regimen. It can also help the patient get some relief from muscle stiffness and joint pain. Occupational therapies are used to improve motor skills. Speech therapies are used to improve the speech and the language barriers developed due to the progression of the disease. It can also help to get rid of the swallowing and the chewing problems developed due to the disease.

Medications: Medicines that are prescribed for PD include: drugs for increasing the dopamine levels and the drugs that can help in controlling the non-motor symptoms.

The most common medicine that is used for treating PD is levodopa, L-dopa. L-dopa helps to replenish the levels of dopamine in the brain. To minimize the side effects of L-dopa, it is usually taken with carbidopa. There can be many other medications to treat the disease like: dopamine agonists to mimic the role of dopamine in the brain, MAO-B inhibitors, COMT inhibitors, and anticholinergic drugs.

Surgery: Surgery is considered in those cases where patients have stopped reacting to the medications. The surgery that is most common and has been approved by the FDA is Deep Brain Stimulation (DBS). It involves implanting an electrode into the parts of the brain, such as subthalamic nucleus (STN) or the globus pallidus interna (GPI). This electrode acts similar to the pacemaker. The electrode is connected to a pulse generator that runs through the wires, and it stimulates the part of the brain that is affected by the disease. It helps to stop movement-related problems of the disease like rigidity, slowness of movement, and tremor.

2.4 AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ALS or Amyotrophic Lateral Sclerosis is a fatal NDD along with AD and PD. It affects the brain and the spinal cord. It is an idiopathic NDD of the human brain [35]. ALS was first described by Charcot in 1874. It is also known as Lou Gehrig's Disease, after the name of it's first patient Lou Gehrig. It is a progressive disorder that involves damage to the motor neurons at different levels [37]. Motor neurons are the ones that run from the brain to the spinal cord and from the spinal cord to various parts of the body. The difference between a healthy neuron and the one affected with ALS has been depicted in figure 4.

"Amyotrophic" refers to the muscular atrophy, weakness and fasciculation that implies it to be the disease of the lower motor neurons [38]. "Lateral Sclerosis" refers to the hardness of the anterior and the lateral columns of the spinal cord [38]. ALS occurs between the age of 40 and 70 years. Usually, the most common age for the occurrence of the disease is 55 years.

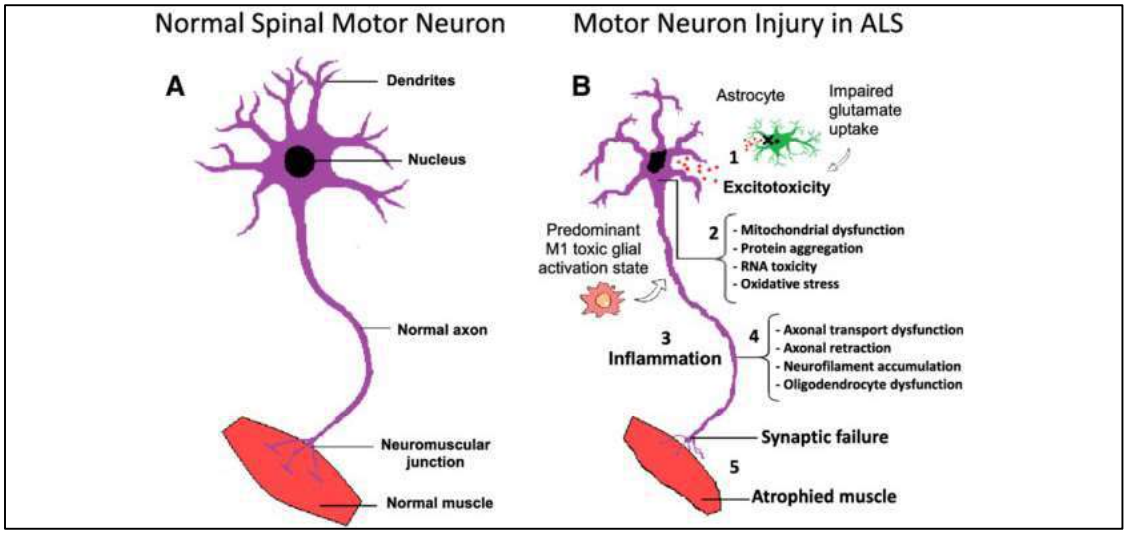

Figure 4: Difference between a healthy neuron and ALS affected neuron [39].

2.4.1 Causes of Amyotrophic Lateral Sclerosis

The causes of ALS are not known. But the studies done by the scientists suggest that both the genetic as well as environmental factors play a role in the development of the disease.

- **Genetics:** In 1993, scientists discovered that the gene *SOD1* was associated with the familial type of ALS. Other genes that can cause the disease are *ALS2, ALS4, VAPB*, *angiogenin*. Certain changes in the processing of the RNA can also lead to the degeneration of the motor neurons, thereby causing the disease. RNA molecules in the body are involved in the synthesis of the proteins and regulating gene expression and

activity. The mutations in the structure and shape of the motor neurons can also play a role in causing the disease.

- **Environmental Factors:** an increased susceptibility to environmental toxins can cause the disease. Unhealthy diet, physical trauma, behavioral and occupational factors can also lead to the disease.

2.4.2 Types of Amyotrophic Lateral Sclerosis (ALS)

There are basically two types of ALS:

- **Sporadic ALS:** The majority of the ALS cases are sporadic. In this type, the disease suddenly occurs at random with no associated environmental risk factor or genetic factors. The family members of the patient with sporadic ALS are at a greater risk of developing the disease.
- **Familia (Genetic) ALS:** about 5 to 10% of the total cases are familial. It is caused, if one of the parents carries the gene responsible for causing the disease. A number of genes have been identifying to cause the disease. But mainly the mutations in the genes *SOD1* and *C90RF2* are known to cause the disease in most of the cases.

2.4.3 Signs and Symptoms of Amyotrophic Lateral Sclerosis

Both the sporadic and the familial ALS are associated with the progressive loss of the motor neurons. But the symptoms depend upon which part of the brain is being affected. Some of the early symptoms are:

- Fasciculations in the arm, shoulder, leg or tongue
- Muscle cramps
- Tight and stiff muscles
- Muscle weakness
- Slurred and nasal speech
- Difficulty in chewing or swallowing
- Tripping or falling
- Persistent fatigue

As the disease progresses, it can lead to paralysis in certain cases. Symptoms can first appear either in the hands or the legs, calling it the “limb onset” ALS or problems in swallowing or speaking, calling it the “bulbar onset” ALS. Regardless of where the symptoms appear first, muscle weakness and atrophy continue to worsen as the disease progresses. People with ALS

can face difficulty in breathing as the muscles of the respiratory system get weakened. They eventually lose the ability to breathe on their own, and hence they depend on the ventilator. Affected individuals also have a greater risk of pneumonia at the later stages.

2.4.4 Treatment of Amyotrophic Lateral Sclerosis

As the disease progresses, it becomes more and more difficult for the patient to breathe and digest food. Till date as such, there is no cure for the diseases. The medications and the therapies that are suggested by the medical professionals can only reduce the symptoms to lead an easy life.

- **Medications:** Till date, Riluzole and Edaravone are the two drugs that have been approved by the FDA to be used for the treatment of the disease. Riluzole reduces the damage done to the motor neurons by decreasing the levels of glutamate, which is a transporter between the nerve cells and the motor neurons. Edaravone reduces the clinical assessment in daily functioning with the people affected by ALS.
  The physicians can also prescribe medications to reduce muscle cramps, depression and the other symptoms related to the disease.
- **Physical Therapy:** It can help the patient to strengthen muscles, improve cardiovascular health, and help fight fatigue and depression. Physical Therapy involves exercises such as walking, swimming, and stationary bicycling.
- **Speech Therapy:** It helps the patients to improve their speaking ability and benefits them by improving their communication ability, which had been affected due to the disease. Computer aids such as computer-based speech synthesizers can also help them.

2.5 HUNTINGTON'S DISEASE (HD)

Huntington's Disease is a complex NDD that affects the CNS [40]. It is a genetic NDD that is passed down from the parents to the child. It caused due to one single defective gene on chromosome 4. This defect is dominant, hence would be transferred over the generations. The disorder was first discovered by George Huntington in the 1800s.

The defective gene causes the build-up of the protein called Huntington, that damages the nerve cells in the brain, thereby causing the disease. During the course of this disease, there is a gradual development of involuntary muscle movements affecting the hands, face, feet, trunk and cognitive impairment.

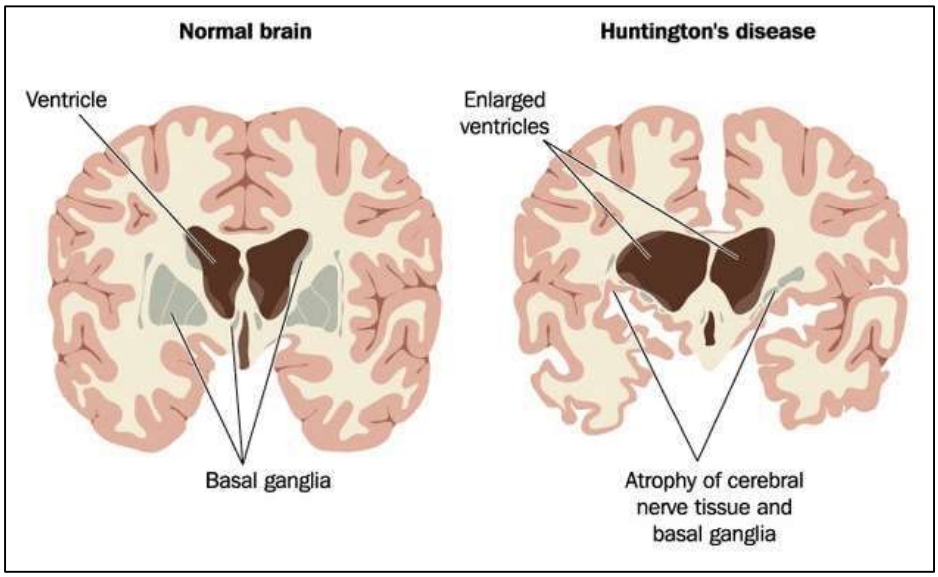

Figure 5: Difference between a healthy brain and HD brain. (Source: Medanta)

2.5.1 Causes of Huntington's Disease

HD is an autosomal dominant inherited disorder. It is caused by mutations of the gene located on the short arm of chromosome 4. This gene provides instructions for making up a protein called huntingtin. The function of this protein is still unknown, but it does play an important role in neurons in the brain.

In this mutation, the gene has a DNA segment known as CAG trinucleotide repeat. This segment is made up of three building blocks, namely, cytosine, adenine and guanine. Usually, a healthy person has 10 to 35 copies of the CAG segment. But in an HD affected individual, the number of copies of this segment is repeated 36 to 120 times, which leads to overproduction and abnormally long segment of the huntingtin protein. The elongated protein is cut into smaller pieces and forms aggregates in the neurons of the brain. The following image shows how an elongated protein is created (figure 6).

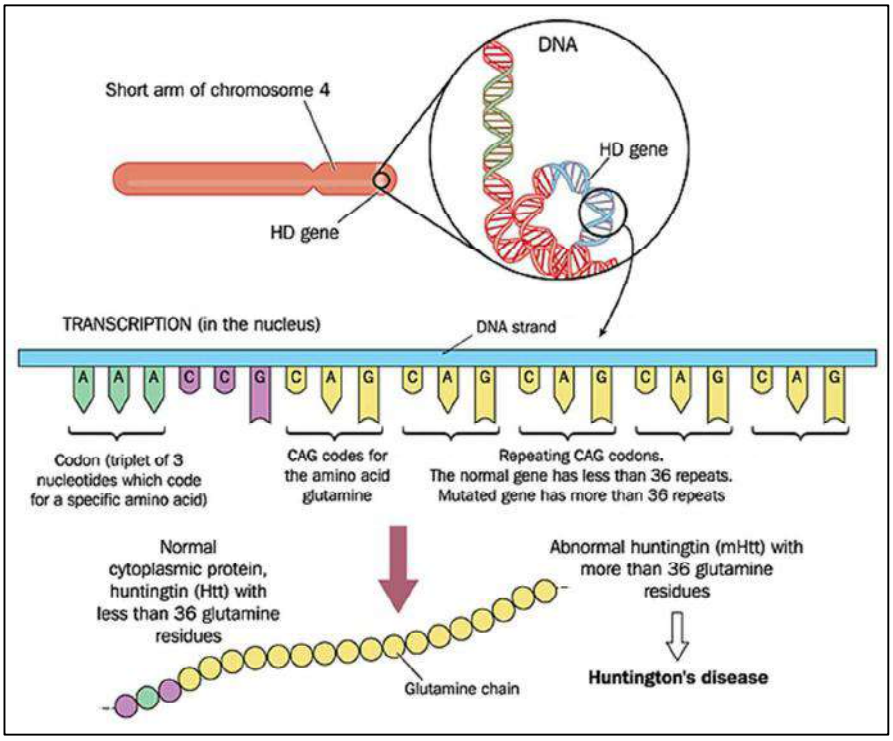

Figure 6: Process of creating an elongated protein. (Source: NIH)

2.5.2 Types of Huntington's Disease

HD is of the following two types:

- **Adult-onset HD:** This condition is the most common. It occurs in people who are in their thirties or forties. Early symptoms can include irritability, depression, small involuntary movements, poor coordination, and trouble making new decisions and learning new things. Individuals with the adult-onset of the disease live for up to 15 to 20 years after the signs and symptoms begin to appear.
- **Juvenile-onset HD:** This condition either begins in childhood or adolescence. It involves movement problems, mental and emotional changes. Additional symptoms of this condition can be drooling, clumsiness, frequent falling, and slurred speech. Seizures occur in 30 to 50% of the children. Their school performance drops. This condition progresses more rapidly than the adult-onset condition, and the patients can live up to 10 to 20 years after the signs and symptoms start appearing.

2.5.3 Signs and Symptoms of Huntington's Disease

The symptoms can start appearing in the 30s or 50s, and in some cases, it can be as early as 2 years of age or as late as 80 years of age. It is characterized by rapid uncontrollable movements such as tics or muscle jerks. It also causes a decline in thinking and reasoning skills, including memory, concentration, and the ability to plan and organize.

The brain changes can lead to depression, sudden mood swings, irritability and uncharacteristic anger. The most common symptom is that the person develops obsessive-compulsive disorder, asking the same questions over and over again. Patients with the disease are at a greater risk of developing pneumonia due to being bedridden all the time and poorly nourished.

2.5.4 Treatment of Huntington's Disease

There is currently no cure for HD. The treatments that are available focus on managing symptoms.

In August 2008, the FDA approved the drug Tetrabenazine for the treatment of involuntary movements associated with HD. This is the only drug that has been approved for the disease.

Other treatments are providing therapies. Occupational therapy to help make everyday tasks easier. Speech and language therapy for communication and feeding problems. Physiotherapy to help the individual with movement and balance.

2.6 INDIAN SPICES AND HERBS

Spices are the dried, pleasantly aromatic parts of the plant. As per the Food and Drug Administration (FDA), spices are: " aromatic vegetable substances, in the whole, broken, or the ground form, whose significant function is seasoning in food rather than nutrition" [5]. Spices are substances that have been used since time immemorial as a food additive for the purpose of flavoring and adding colors, and sometimes as preservatives to kill the harmful bacteria [41]. Spices have been used for purposes such as medicine, religious, cosmetics, vegetables or perfumery, other than flavoring [6]. A herb and a spice are different from each other in the way that: spices are obtained from all the parts of the plants other than the leaves, whereas the herbs can be obtained only from the leaves [42].

The source of most of the herbs and the spices lies in the Mediterranean countries, especially the Middle East or Asia, and they have been used since time immemorial in the day-to-day lives, specifically in the Indian kitchens [43]. Herbs and spices have played, and still continue

to play, important roles as flavoring agents, food preservatives and medicines. Over the past few decades, a lot of research into the health benefits of these has increased significantly, due to their ability to treat various chronic diseases [5]. Their potential health benefits include conferring protection for cancer, neurodegenerative diseases, cardiovascular diseases, obesity, and type 2 diabetes [44], [45], [46], [47], [48].

The positive health effects of herbs and spices is towards preventing the chronic diseases. They prevent the diseases due to the direct action of their constituent phytochemicals (particularly polyphenols) targeting specific receptors or enzymes involved in various anti-inflammatory pathways or immune responses [49]. Herbs and spices (especially, the dried form) contain a high content of polyphenols and other physiologically active phytochemicals [7].

2.7 SECONDARY METABOLITES

The sum of all the biochemical processes that take place in an organism is called metabolism. The main primary metabolites of plants are produced through the fundamental metabolic pathways such as Glycolysis, Calvin Cycle, Krebs Cycle, etc. The main primary metabolites are carbohydrates, lipids, proteins, and nucleic acids. They are found in plants and their main function is to help the plants to survive [50].

Plant secondary metabolites are considered to have an irrelevant fundamental role in preserving the life processes in plants, but they do play a significant role in the interaction of the plant with its surrounding environment for adaptation and defense.

Secondary metabolites, also known as natural products, that are the products of the metabolism process not essential for normal growth, development or reproduction of an organism, whereas they are necessary for meeting the requirements of the organisms. They help the organisms in surviving the interspecies competition, providing defensive mechanisms and also facilitating the reproductive process. They also accord specific odors, colors and tastes in plants [51].

Secondary Metabolites are inimitable sources for pharmaceuticals, food additives, flavors, and industrially important pharmaceuticals [52], and they also have significant practical applications in medicinal, nutritive, and cosmetic purposes besides playing a role in plant stress physiology for adaptation [53]. Over 2,14,000 secondary metabolites are known till now, and they have been classified according to their rich diversity, structure, function, and biosynthesis. Secondary Metabolites are basically categorized into the following classes:

1. Terpenoids and steroids

2. Fatty acid-derived substances and polyketides
3. Alkaloids
4. Non-ribosomal polypeptides
5. Enzyme cofactors

2.7.1 Role of Secondary Metabolites

Among the Plantae Kingdom, the angiosperms are spread over the majority of the terrestrial surface, contributing exceptionally to the biomass in terms of their volume and weight in comparison to the other life forms combined together [54].

Plants have to overcome a number of challenges, like seed dispersal, fluctuations in the supply of the basic nutrients for their survival and their interdependency with the other organisms and herbivores in their proximate environment. Hence, during the course of evolution, the plants have developed numerous secondary biochemical pathways allowing them to synthesize innumerable chemicals while responding to their specific environmental stimuli [54][55]. They do have any substantial role in the primary metabolic requirements of the plants, rather they increase the overall surviving ability of the plants and in overcoming the challenges when interacting with their immediate environment [56].

There are various other roles of secondary metabolites such as they act as a general host for various protective roles thereby helping the plants to defend themselves from the microorganisms. They also help the plant to manage the inter-plant relationships [57]. Here, it is clear that the primary role of the secondary metabolites is to act as a feeding deterrence, and because of this these phytochemicals can be toxic to the potential herbivores, by interacting directly with the CNS and the PNS of the herbivore [58]. In this regard, secondary metabolites act as antagonists or agonists of neurotransmitter systems [59][60] or form structural analogs of the endogenous hormones [61].

In order to survive, plants also have to foster innumerable symbiotic relationships. Another role of the secondary metabolites lies here that is attracting the pollinators by the various colors and scents. Thereby providing an attractive chemical for the predator resulting in the synthesis and release of a cocktail of phytochemicals to attract the natural predators of the herbivore [57].

2.8 FLAVONOIDS

In recent times, there has been an interest in the potential role of the flavonoids to modulate neuronal function and prevent age-related neurodegeneration. Flavonoids refer to a class of secondary metabolites that have a polyphenolic structure. They are the most abundant in fruits, vegetables and plant tissues [62], and thus, they are included in the diet of the humans [63]. One of the most remarkable properties of flavonoids is that they have the good antioxidant potential [64]. Thus, they have pharmacological importance as therapeutic agents. The physiological activity of the flavonoids has been reported to be due to their structure and geometry [65].

Based on their chemical structure, they have been divided into flavonols, flavanols, flavones, flavanones, isoflavones, anthocyanidins, and chalcones. Till date, 9000 flavonoids have been reported, all present in fruits and vegetables. All the flavonoids share a common structure in the sense that they have two aromatic rings (A and B), which are connected by three carbon atoms, thereby forming an oxygenated heterocycle (ring C) (figure 7). The variations in their saturation of the basic flavan system, their alkylation, and/or glycosylation and the hydroxylation pattern of the molecules form the basis of their subcategories that have been discussed above.

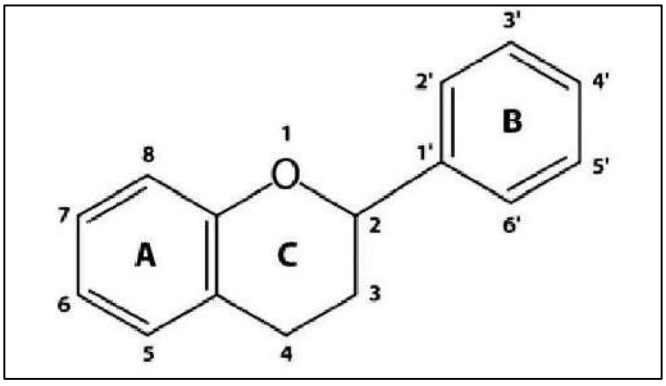

Figure 7: Basic Flavonoid Structure [66]

2.8.1 Properties of Flavonoids

Flavonoids possess a number of properties that attribute to their use in the treatment of various diseases. Some of the properties are described below:

1. They possess numerous biological effects such as antiviral, anti-inflammatory, anti-tumor, anti-allergic, antiplatelet, and antioxidant activities [67].

2. They can easily cross the Blood Brain Barrier (BBB) and may show neuropharmacological activities at the molecular level, thereby manipulating the protein function and gene expression [13].
3. The performance of the spatial memory improves due to the improvement in the brain-derived neurotrophic factor (BNDF), which is affected due to the dietary intake of the flavonoids [68].
4. Flavonoids can regulate the immune system of the brain, and thus attenuate the neuroinflammation by inhibiting the production of nitric oxide and cytokines that are produced by the activated microglia [69].
5. They also possess the ability to modulate NFκB and mitogen-activated protein kinases (MAPKs) signaling pathways and also attenuate the function of COX-2, IL-6, IL-1β, and TNF-α [70].

The above properties of the flavonoids show that they can be a good fit for the NDD profile, and in a process dependent on the suppression of the lipid peroxidation, inhibition of inflammatory mediators, modulation of gene expression, and activation of antioxidant enzymes, they can protect the neurons from degenerating and thereby preventing the neurodegeneration [71], [72].

2.8.2 Classes and Sources of Flavonoids

Due to the multiple effects of the flavonoids, scientists have been drawn towards the investigation of the neuroprotective role of the flavonoids. The various classes and their sources of the flavonoids have been discussed in table 2.

Table 2: Classes and Sources of Flavonoids

Classes	Flavonoids	Common Sources
Flavonols	Rutin, Quercetin, Kaempferol, Myricetin	Leeks, onions, kale, broccoli, apples, cherries, berries, red wine, tea
Flavanols	Catechin, Epicatechin, Epigallocatechin, Epigallocatechin gallate (EGCG)	Green tea, black tea, blueberries, tea, cocoa, grapes, chocolate
Isoflavones	Genistein, Daidzein, Glycetin, Formanantine	Legumes, soy-beans, soy products,
Flavones	Luteolin, Apigenin, Acacetin,	Parsley, celery, apple skins, chrysanthemum flowers, cabbage, peppers, carrot
Flavonones	Hesperetin, Naringin, Isoxanthohumol, Taxifolin	Citrus fruits, tomatoes, grapefruits

Anthocyanidins	Cyanidin, Malvidin, Pelargonidin, Delphinidin	Red wine, berry fruits, cherries, grapes, kidney beans

2.8.3 Flavonoids: Treatment in Neurodegeneration

NDDs are characterized by the changes in the structural and pathological conditions. Thus, innumerable targets and methods with better efficacy are needed for their treatment. Flavonoids affect the cell system by modulating the activity of the various metabolic pathways and thereby reducing the cognitive decline and neuronal dysfunction [73] . They can also prevent or delay the onset of the NDD at their desired doses and concentrations.

3. METHODOLOGY

Virtual screening of the pharmacophore properties was done of the compounds, and their structures were obtained. The ligand structures were obtained in the ".sdf" format, and the protein structures were obtained in the ".pdb" format. Further, their docking was carried out against the well-known target of the diseases.

3.1 Software's and Databases

To conduct the study the following Databases were used to obtain the data:

- PubChem (https://pubchem.ncbi.nlm.nih.gov/) [74]
- ChEBI (https://www.ebi.ac.uk/chebi/) [75]
- DrugBank (https://www.drugbank.ca/) [76]
- RCSB PDB (https://www.rcsb.org/) [77]
- FooDB (https://foodb.ca/)

The software's that were used to predict the results are:

- Molsoft L.L.C.: Drug-Likeness and Molecular Property Prediction (https://molsoft.com/mprop/mprop.cgi)
- Molinspiration software (https://www.molinspiration.com/cgi-bin/properties)
- pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]
- PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]
- Open Babel [80]
- CASTp (Computed Atlas of Surface Topography of Proteins) (http://sts.bioe.uic.edu/castp/index.html?1ycs) [81]
- AutoDock Vina in PyRx [82]

3.2 Collection of data of Indian spices and their flavonoids

The list of the Indian Spices was created using the literature survey and their phytochemical constituents were summarized using several publications [83], [84], [85].

3.3 Selective prediction of the Molecular Properties

The molecular properties of the flavonoids were obtained using the software Molinspiration software (https://www.molinspiration.com/cgi-bin/properties). In this software, SMILES were used as the input format. The SMILES of the compounds were obtained from PubChem/ChEBI

database. The RO5 is an important criterion to predict that the compound can be used as a potential drug in which the log P should not exceed 5, the molecular weight should not be more than 500 daltons, and the number of hydrogen bond acceptors (HBA) should be less than 10 and the hydrogen bond donors (HBD) to be less than 5. Those flavonoids that violated the rule were omitted from further analysis.

3.4 Selective prediction of the Drug Likeness Properties

The Drug Likeness properties were obtained using the software Molsoft L.L.C.: Drug Likeness and Molecular Property Prediction (https://molsoft.com/mprop/mprop.cgi). The SMILES were used as the input, which were obtained from the PubChem/ChEBI database. The value of the drug-likeness score has to be between 0 and 1. Those flavonoids that had the drug-likeness score less than 0 were not used for the further analysis.

3.5 Selective Prediction of the BBB value

The BBB (Blood Brain Barrier) is an important factor for a compound to be used as a drug as it must cross the BBB readily. The BBB value was calculated using the PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]. The criteria to differentiate the compounds was whether they had high, medium or low permeability. If the

- logBB>0.3, high permeability;
- logBB lies between 0.3 and -1.0, medium permeability;
- logBB<-1.0, low permeability.

Those flavonoids that showed high permeability were used for further analysis.

3.6 Predicting the Pharmacokinetic Properties

The pharmacokinetic profile of a compound describes its ADME (absorption, distribution, metabolism, excretion and toxicity) properties. The prediction of the ADME properties was done in order to increase the success rate of the compound as a drug. The ADME properties were calculated for the flavonoids. The properties include Aqueous solubility, Blood Brain Barrier level, CYP 2D6, and Plasma Protein Binding Level. The properties were predicted using the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78], in which the SMILES were used as an input obtained from the PubChem/ChEBI database.

Analysis of pharmacokinetic profiles of the ligands is an imperative assortment before heading on towards the preclinical and the clinical assessments. They help us to pinpoint whether the

ligands have the potential to act as druggable molecules for the treatment of the various disorders. The outcomes define the conspicuous functionalities of the structures of the ligands [86].

Generally, only the unbound drug is available for diffusion or transport across the cell membranes, and also for an interaction with a pharmacological target. As a result, degree of plasma protein binding of a drug influences not only the drug's action but also its disposition and efficacy. If the Plasma Protein Binding (%PPB),

- PPB more than 90%, chemical strongly bound
- PPB less than 90%. Chemical weakly bound

3.7 Toxicity Determination of the Compounds

The toxicity of the compounds was determined with the help of the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]. The toxicity of the FDA approved drugs of the respective diseases was compared with the toxicity of the screened flavonoids. The properties include rat LD50, AMES toxicity, Maximum Tolerated Dose, and Hepatotoxicity.

The toxic potency of a compound is an important parameter, hence for this purpose lethal dosage (LD50) is a standard measurement. The amount of a compound given all at once and that causes the death of 50% of the group of test animals, is called LD50.

The AMES test is used to assess the compound's mutagenic potential using bacteria. If a compound is tested positive, that means it is mutagenic having the carcinogenic potential.

To get an estimate of the toxic dose threshold of the chemicals in humans, it is provided by the maximum recommended tolerated dose (MRTD). If MRTD $\leq$ 0.477 log (mg/kg/day), it is considered low and if MRTD > 0.477 log (mg/kg/day), it is considered high.

Drug-induced liver injury is one of the major concerns for developing a drug. With the help of hepatotoxicity, one can predict whether a compound is affecting the normal functioning of the liver or not.

3.8 Analysis of the 3-D Structures of the target Proteins of the disease

The target proteins that were chosen were Acetylcholinesterase (AChE, PDB ID: 4PQE) for AD, Dopamine (DA, PDB ID: 6VMS) for PD, Synaptic Vesicular Membrane Transport (VAT-1, PDB ID: 6K9V) for HD, and Soluble Carrier Family 22 Member 6 (S22A6, PDB ID: 2KBI) for ALS. Only these target proteins were chosen because they were the targets for the FDA

approved drugs and hence useful for the study in order to compare the inhibitory activities of the flavonoids against the similar drug targets. The 3-D structure of the target proteins was obtained from the RCSB PDB (Protein DataBank, https://www.rcsb.org/) [77] in the PDB format. These proteins would be acting as receptors for the docking analysis.

3.9 Identification of the 3-D Structures of the Ligands

The ligands that were chosen were of two types, conventional drugs approved by the FDA for the treatment of the various NDDs and the screened flavonoids.

(a) The 3-D structure of these drugs was obtained from the DrugBank (https://www.drugbank.ca/) [76]. The structure of the drugs was obtained in the SDF format and then was converted into the PDB format using Open Babel [80].

(b) The 3-D structure of the screened flavonoids was obtained from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) [74] and ChEBI (Chemical Entities of Biological Interest, https://www.ebi.ac.uk/chebi/) [75]. The structures were obtained in the SDF format and then were converted into the PDB format using the software Open Babel [80].

3.10 Analysis using Molecular Docking

Molecular Docking is a striking scaffold to understand the various drug bio-interactions [87]. It is the mechanistic study where a ligand is placed into the preferred binding site of the receptor to form a stable complex in a non-covalent fashion [87]. The docking analysis was performed using the AutoDock Vina in PyRx [82].

3.11 Bioactivity Prediction of the Flavonoids

The flavonoids that showed higher binding affinity than the FDA approved drugs with their target proteins were broken down with the help of the Molinspiration software (https://www.molinspiration.com/cgi-bin/properties) to check whether the compounds were bioactive or not.

The bioactivity scores include the activity of the compounds towards GPCR ligands, particle channel modulators, kinase inhibitors, molecular receptor ligands, protease inhibitors and other enzyme targets.

The compounds are said to be

- bioactive, if bioactivity score >0;

- moderately active, if bioactivity score lies between -5.0 to 0;
- and inactive, if the bioactivity score <-5.0.

3.12 Prediction of the Antioxidant Property of the Flavonoids

The flavonoids that showed the low binding affinity with the proteins were checked for their antioxidant properties using the PASS Server (http://www.pharmaexpert.ru/passonline/) [88]. The chemical structure in PASS is described by the descriptors called Multilevel Neighbourhoods of Atoms (MNA). For each prediction, two probabilities, Pa and Pi are calculated based on the statistics of the MNA descriptors. Pa and Pi values range between 0.000 to 1.000, thereby indicating whether the compound is active or inactive, respectively [89]. The input for the PASS server was the SDF files of each of the flavonoids. It predicted the antioxidant activity in general and also the other activities that were related to it. Pa value was chosen as more than 0.7 (cut-off value) [90].

4. RESULTS AND DISCUSSION

4.1 Database of the Indian Spices and their flavonoids

The Indian spices that were chosen are the ones that have been used routinely. The database (table 3) of the Indian spices and their chemical constituents (flavonoids) was created with the help of several publications. A total of 24 Indian spices were taken into consideration.

Table 3: Database of the Indian Spices and their chemical constituents (flavonoids).

S.No.	Scientific Name	Common Name	Flavonoids Present
1.	*Curcuma longa*	Turmeric (Haldi)	Curcumin
2.	*Thymus vulgaris*	Thyme (Ajwain)	Apigenin, Chrysin, Luteolin, Diosmetin
3.	*Coriandrum sativum*	Coriander (Dhania)	Acacetin, Quercetin, Kaempferol
4.	*Ocimum basilicum*	Basil (Tulsi)	Quercetin, Catechin, Kaempferol, Rutin, Luteolin
5.	*Allium sativum*	Garlic (Lehsun)	Kaempferol, Myricetin
6.	*Nigella sativa*	Nigella (Kalonji)	Quercetin, Kaempferol
7.	*Azadirachta indica*	Neem	Quercetin, Kaempferol, Melicitrin
8.	*Sesamum indicum*	Sesame (Til)	Kaempferol, Naringenin, Apigenin, Quercetin, Myricetin, Luteolin, Rutin, Epicatechin
9.	*Myristica fragrans*	Nutmeg (Jaiphal)	Myricetin
10.	*Zingiber officinale*	Ginger (Adrak)	Quercetin
11.	*Syzygium aromaticum*	Cloves (Laung)	Kaempferol, Luteolin
12.	*Elletaria cardamomum*	Cardamom (Elaichi)	Quercetin, Kaempferol, Luteolin
13.	*Capsicum annuum*	Chilli Pepper (Capsicum)	Quercetin, Kaempferol, Luteolin, Catechin, Epicatechin, Rutin, Myricetin, Apgenin
14.	*Cinnamonum verum*	Cinnamon (Dalchini)	Gossypin, Gnaphalin, Hesperidin, Hibifolin, Hypolaetin, Quercetin
15.	*Trigonella foenum-graecum*	Fenugreek (Methi)	Vitexin, Tricin, Naringenin, Quercetin
16.	*Solanum lycopersicum*	Tomato	Naringenin, Rutin
17.	*Allium cepa*	Onion	Quercetin, Kaempferol, Isorhamnetin, Myricetin, Cyanidin
18.	*Laurus nobilis*	Bay Leaf (Tejpatta)	Kaempferol, Quercetin, Apigenin, Luteolin, Isorhamnetin
19.	*Cuminum cyminum*	Cumin	Kaempferol, Quercetin
20.	*Syzygium aromaticum*	Cloves	Kaempferol, Quercetin
21.	*Brassica*	Mustard	Isorhamnetin, Kaempferol, Quercetin
22.	*Crocus sativus*	Saffron	Quercetin, Kaempferol, Myricetin, Naringenin, Taxifolin, Tamarixetin, Isorhamnetin
23.	*Foeniculum vulgare*	Fennel seeds	Rutin, Quercetin, Kaempferol, Eriodictyol
24.	*Magnifera indica*	Amchoor (Dry mango powder)	Epicatechin, Catechin, Apigenin, Luteolin, Quercetin, Kaempferol, Myricetin

4.2 Prediction of Molecular Properties

A total of 27 flavonoids were obtained from the above-created database. The molecular properties of the flavonoids were obtained using the Molinspiration software (https://www.molinspiration.com/cgi-bin/properties). For calculating these properties, the canonical SMILES of the flavonoids were obtained from the PubChem/ChEBI database, and they were checked for Lipinski's Rule of Five (RO5). The results are depicted in table 4.

Table 4: Molecular Properties of the Flavonoids obtained using the Molinspiration Software.

S.No.	Flavonoid	milogP	TPSA	n atoms	HBA	HBD	*n* rotb	MW	Volume
1.	Catechin	1.47	110.38	21	6	5	1	290.27	261.53
2.	Epicatechin	1.37	110.38	21	6	5	1	290.27	261.13
3.	Luteolin	1.86	111.13	21	6	4	1	286.24	272.86
4.	Apigenin	1.89	90.90	20	5	3	1	270.24	260.14
5.	Tangeretin	3.71	76.36	27	7	0	6	372.37	385.27
6.	Naringenin	1.75	86.99	20	1	5	3	272.25	251.12
7.	Myricetin	1.08	151.59	23	8	6	1	318.24	292.39
8.	Isorhamnetin	2.35	120.36	23	7	4	2	316.26	301.71
9.	Chrysin	2.94	70.67	19	4	2	1	254.24	216.03
10.	Rutin	-1.06	269.43	43	16	10	6	610.52	496.07
11.	Acacetin	3.00	79.90	21	5	2	2	284.27	241.58
12.	Diosmetin	2.28	100.13	22	6	3	2	300.27	249.59
13.	Kaempferol	1.70	111.13	21	6	4	1	286.24	268.99
14.	Gossypin	-0.62	230.73	34	13	9	4	480.38	380.22
15.	Quercetin	1.63	131.36	22	7	5	1	302.24	240.08
16.	Vitexin	1.63	181.05	31	10	7	3	432.28	391.88
17.	Gnaphalin	1.57	89.27	26	6	1	2	360.41	320.37
18.	Hesperidin	-0.55	234.30	43	15	8	7	610.57	511.79
19.	Hibifolin	-0.75	247.80	35	14	9	4	494.36	382.40
20.	Hypolaetin	1.71	131.35	22	7	5	1	302.24	240.08
21.	Tricin	2.30	109.36	24	7	3	3	330.29	275.14
22.	Taxifolin	0.71	127.44	22	7	5	1	304.25	246.32
23.	Tamarixetin	1.99	120.36	23	7	4	2	316.26	257.61
24.	Curcumin	2.30	93.07	27	6	2	8	368.38	332.18
25.	Eriodictyol	1.63	107.22	21	6	4	1	288.25	238.28
26.	Pelargonidin	-0.26	92.08	20	5	4	1	271.25	226.79
27.	Cyanidin	-0.75	112.31	21	6	5	1	287.25	234.81

The following violated one or more RO5: Myrictein had HBD >5; Rutin had HBA >10, HBD >5 and the TPSA >200; Gossypin had HBA >10, HBD >5 and the TPSA >200; Hesperidin had HBA >10, HBD >5 and the TPSA >200; Vitexin had HBD >5; and Hibifolin had HBA >10, HBD >5 and the TPSA >200

If any of the RO5 is violated, it means that the compound is not suitable to be used in the form of a drug. Excluding these 6 flavonoids, the rest 21 were then used for further analysis.

4.3 Drug-Likeness Score Prediction

The Drug Likeness score was predicted using the Molsoft L.L.C.: Drug-Likeness and molecular property prediction (https://molsoft.com/mprop/mprop.cgi). The value of the drug-likeness score usually lies between 0 and 1. The drug-likeness score of the flavonoids has been depicted in table 5.

Table 5: Drug-Likeness Score of the Flavonoids obtained using Molsoft L.L.C.: Drug-likeness and molecular property prediction software.

S.No.	Flavonoid	Drug-Likeness Score
1.	Catechin	0.64
2.	Epicatechin	0.64
3.	Luteolin	0.38
4.	Apigenin	0.39
5.	Tangeretin	-0.56
6.	Naringenin	0.82
7.	Isorhamnetin	0.39
8.	Chrysin	0.29
9.	Acacetin	0.29
10.	Diosmetin	0.06
11.	Kaempferol	0.50
12.	Quercetin	0.52
13.	Gnaphalin	-0.19
14.	Hypolaetin	0.25
15.	Tricin	-0.08
16.	Taxifolin	1.00
17.	Tamarixetin	0.16
18.	Curcumin	-0.82
19.	Eriodictyol	0.96
20.	Pelargonidin	0.51
21.	Cyanidin	-0.58

From the table, it is clearly visible that Tangeretin, Gnaphalin, Tricin, Curcumin, and Cyanidin have the negative drug-likeness score, hence they cannot be used in the form of a drug. Now, we have only 16 flavonoids available that would be used for further analysis.

4.4 Selective Prediction of BBB Value

The BBB permeability value of the 16 flavonoids was predicted using the PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]. The criteria to differentiate the compounds was whether they had high, medium or low permeability. The predicted values of the screened flavonoids have been depicted in table 6.

Table 6: BBB Permeability Value of the Flavonoids obtained using PreADMET Server.

S.No.	Flavonoid	logBBB Value	Permeability (High/Medium/Low)
1.	Catechin	0.39413	High
2.	Epicatechin	0.394913	High
3.	Luteolin	0.367582	High
4.	Apigenin	0.565113	High
5.	Naringenin	0.59697	High
6.	Isorhamnetin	0.0580929	Medium
7.	Chrysin	0.93256	High
8.	Acacetin	0.150309	Medium
9.	Diosmetin	0.201086	Medium
10.	Kaempferol	0.286076	Medium
11.	Quercetin	0.372765	High
12.	Hypolaetin	0.211296	Medium
13.	Taxifolin	0.166964	Medium
14.	Tamarixetin	0.127547	Medium
15.	Eriodictyol	0.380271	High
16.	Pelargonidin	0.53039	High

Only those flavonoids that showed logBBB value higher than 0.3 were chosen for further analysis, as according to the criteria mentioned in the materials and methodology, they were readily (high) able to cross the blood brain barrier. The flavonoids that were readily permeable were, Catechin, Epicatechin, Luteolin, Apigenin, Naringenin, Chrysin, Quercetin, Eriodictyol, and Pealrgonidin (total of 9 flavonoids for further analysis).

4.5 Pharmacokinetic Properties Prediction of the Screened Flavonoids

The 9 flavonoids obtained after the screening procedure, were then checked for their pharmacokinetic properties. The pharmacokinetic properties were obtained using the pkCSM

server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]. The properties described are Water solubility, CYP 2D6 (acts as an inhibitor or non-inhibitor), and Plasma Protein Binding efficacy. The pharmacokinetic properties of the screened flavonoids have been depicted in table 7.

Table 7: Pharmacokinetic Properties of the Flavonoids obtained using the pkCSM Server.

S.No.	Flavonoid	Water solubility (log mol/L)	CYP 2D6 (Yes/No)	% PPB
1.	Catechin	-3.036	No	100.000000
2.	Epicatechin	-3.101	No	100.000000
3.	Luteolin	-3.02	No	99.717233
4.	Apigenin	-2.989	No	97.253409
5.	Naringenin	-3.24	No	100.000000
6.	Chrysin	-3.022	No	95.095683
7.	Quercetin	-3.085	No	93.236103
8.	Eriodictyol	-3.505	No	100.000000
9.	Pelargonidin	-3.399	No	100.000000

These results show that the flavonoids possess good pharmacokinetic properties and also, they satisfy all the parameters to be taken over as a good drug. Hence, these flavonoids can be used for molecular docking analysis.

4.6 Toxicity Determination of the Bioactive Compounds

The toxicity of the screened flavonoids was determined using the pkCSM online tool. With the help of this server, their LD50 (lethal dosage 50), AMES toxicity, and Hepatotoxicity values were determined.

The determined values of the various toxicity tests for the FDA approved drugs are shown in table 8A, and that for the flavonoids are shown in table 8B.

Table 8A: Determined values of the toxicity tests of the FDA approved drugs.

S.No.	Drug	Disease	LD50 value	AMES Test	Hepatotoxicity
1.	Aricept	AD	2.999	No	No
2.	Exelon	AD	2.893	No	No
3.	Razadyne	AD	3.402	No	No
4.	Levodopa	PD	2.027	No	Yes
5.	Apomorphine	PD	2.419	Yes	No
6.	Bromocriptine	PD	3.268	No	No
7.	Pramipexole	PD	2.482	Yes	No

8.	Ropinirole	PD	2.573	No	No
9.	Rotigotine	PD	2.718	No	Yes
10.	Carbidopa	PD	2.417	Yes	Yes
11.	Tetrabenazine	HD	2.572	No	Yes
12.	Deutetrabenazine	HD	2.482	Yes	No
13.	Riluzole	ALS	2.443	No	Yes
14.	Edaravone	ALS	2.219	No	No

Table 8B: Determined values of the toxicity tests of the screened flavonoids.

S.No.	Flavonoid	LD50 value	AMES Test	MRTD	Hepatotoxicity
1.	Catechin	2.513	No	0.506	No
2.	Epicatechin	2.057	Yes	0.41	No
3.	Luteolin	2.285	No	0.872	No
4.	Apigenin	2.327	No	0.713	No
5.	Naringenin	2.132	No	0.372	No
6.	Chrysin	2.486	Yes	0.183	No
7.	Quercetin	2.449	No	0.956	No
8.	Eriodictyol	1.866	Yes	0.601	No
9.	Pelargonidin	2.433	No	0.787	No

From the above table 8B, it can be clearly seen that Epicatecin, Chrysin, and Eriodictyol are mutagenic and hence they are toxic to the human body and cannot be used for further analysis. Many synthetic drugs fail this toxicity determination test. Hence it is necessary for every compound to pass this test in order to be used as a drug.

The LD50 values of all the screened flavonoids have been compared with the LD50 values of the FDA approved drugs with the help of a graph (figure 8), depicting that the flavonoids have better LD50 values as compared to the LD50 values of the FDA approved drugs.

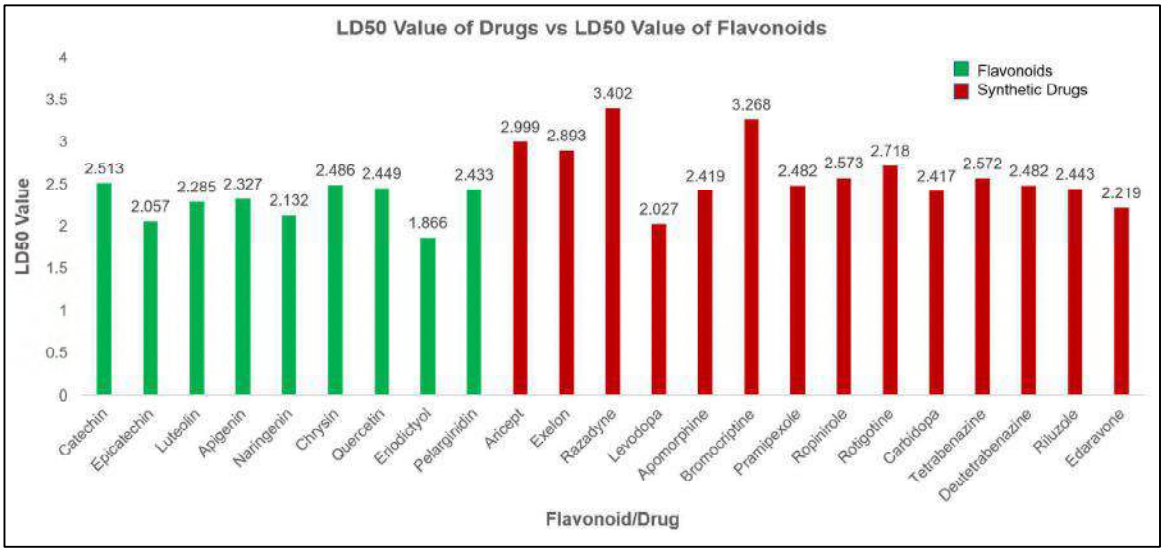

Figure 8: Graph depicting the LD50 values of the FDA approved drugs for the various NDDs and the screened flavonoids.

From the above graph, it can be clearly stated that the screened flavonoids show better LD50 values in comparison to any of the drugs approved for the respective diseases.

4.6 Analysis of the 3-D Structures of the Proteins

The protein that were chosen are:

- Acetylcholinesterase (AChE) for AD
- Dopamine (DA) for PD
- Soluble Carrier Family 22 Member 6 (SCN) for ALS
- Synaptic Vesicular Membrane Transport (SCN) for HD

The proteins were chosen on the basis of the targets of the drugs that were approved by the FDA for the respective diseases. All these targets are the potential targets of their respective diseases. The structures of the proteins were obtained from RCSB PDB (https://www.rcsb.org/) [77]. They were obtained in the ".pdb" format. The structures, along with their PDB IDs, are depicted in figure 9.

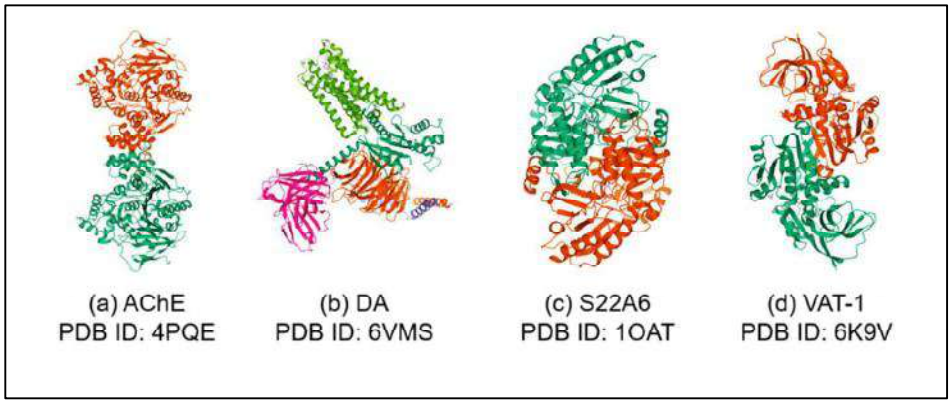

Figure 9: 3-DStructures of the protein targets obtained from the RCSB PDB chosen for the study. 3-D structure of (a) AChE for AD, DA for PD, (c) S22A6 for ALS, and (d) VAT-1 for HD.

4.7 Analysis of the 3-D Structures of Ligands

The ligands were of two types: the conventional drugs that were approved by the FDA and the natural compounds.

The conventional drugs that were approved by the FDA:

- Aricept, Exelon and Razadyne for AD (figure 10A).
- Levodopa, Apomorphine, Bromocriptine, Pramipexole, Ropinirole, Rotigotine, and Carbidopa for PD (figure 10B).
- Riluzole and Edaravone for ALS (figure 10C).
- Tetrabenazine and Deutetrabenazine for HD (figure 10D).

The structures of conventional drugs were obtained from the DrugBank (https://www.drugbank.ca/) [76].

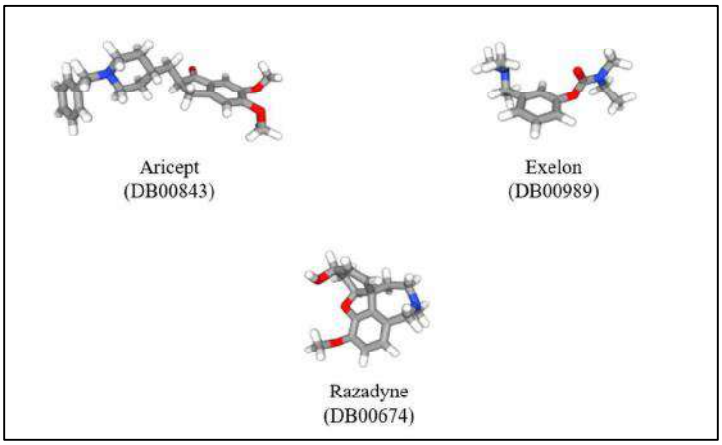

Figure 10A: 3-D Structures of FDA approved drugs for AD.

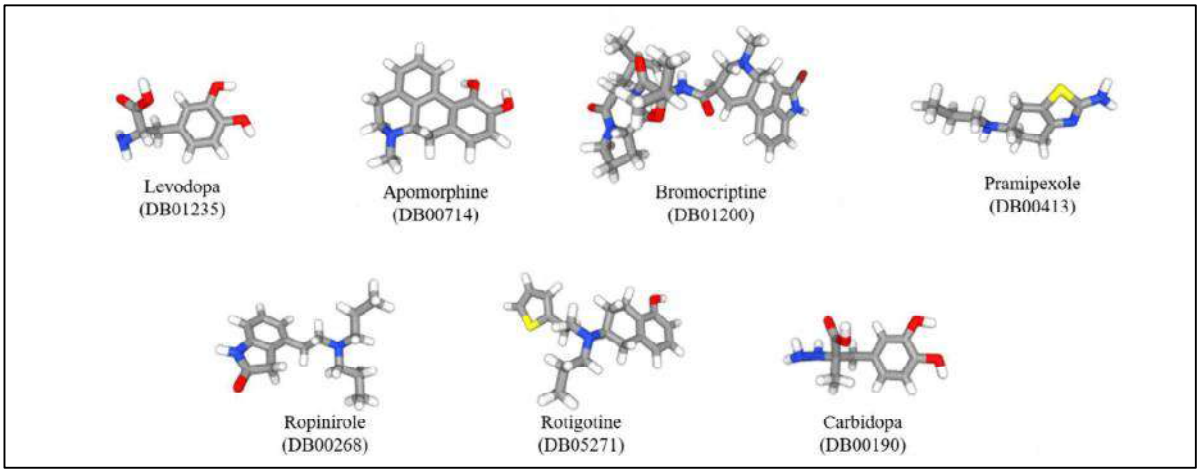

Figure 10B: 3-D Structure of FDA approved drugs for PD.

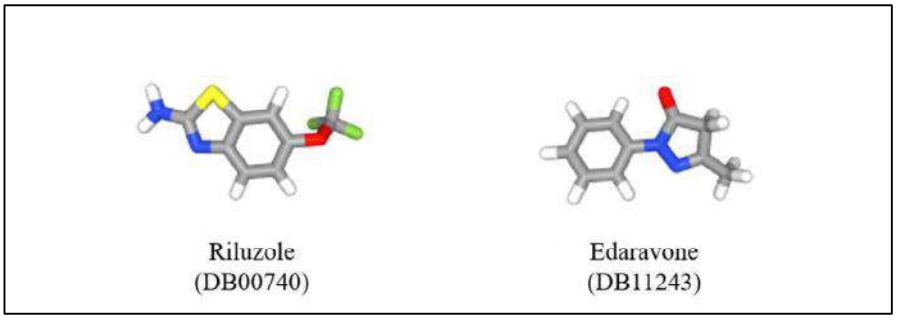

Figure 10C: 3-D Structure of FDA approved drugs for ALS.

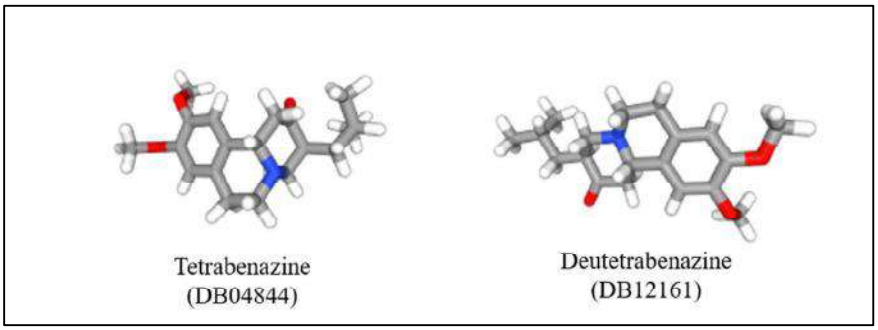

Figure 10D: 3-D Structure of FDA approved drugs for HD.

A total of 06 screened flavonoids (figure 11) were chosen for the molecular docking analysis. Their structures were obtained from the ChEBI (Chemical Entities of Biological Interest, https://www.ebi.ac.uk/chebi/) [75]. The flavonoids that were not available on the ChEBI database, their structures were obtained from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) [74].

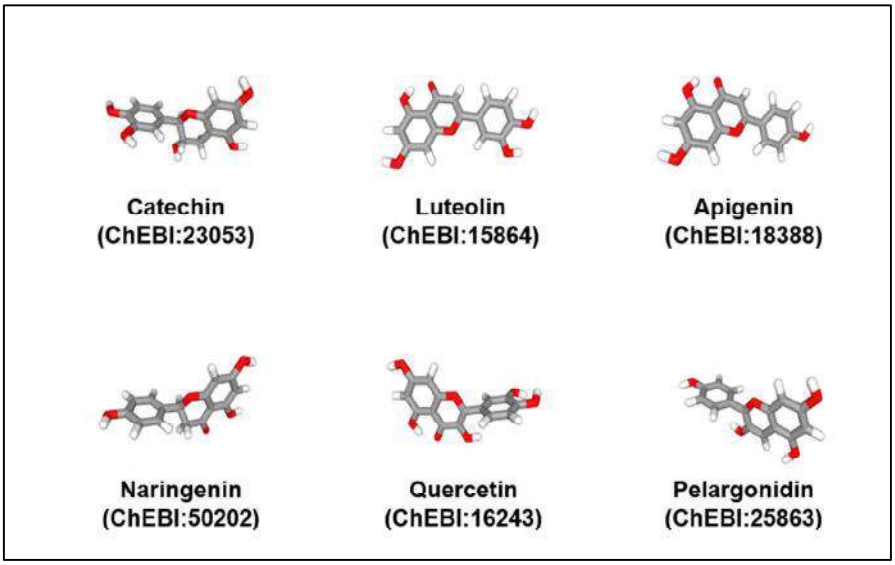

Figure 11: 3-D structure of the six screened flavonoids obtained from the ChEBI (Chemical Entities of Biological Interest) and in some from the PubChem.

4.8 Molecular Docking Studies

Molecular docking is a molecular modelling approach which involves the interaction of two or more molecules and provides stability to the resultant structure [91]. The docking analysis was carried out using the AutoDock Vina in PyRx. The ligands were docked against protein receptors, which were the most potent targets of their respective diseases. The conventional drugs were all protein inhibitors that have been approved by the FDA for the treatment of the respective diseases, and after the analysis obtained from the pharmacokinetics, it can be said that the flavonoids also behaved in a similar way as that of the conventional drugs. Hence, they were also docked against protein targets.

4.8.1 Alzheimer's Disease (AD)

The receptor chosen was AChE. It was docked against the conventional drugs approved by FDA that are Aricpet, Exelon, and Razadyne; and the thirteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The following binding affinities were obtained (table 9):

Table 9: Binding Affinity (kCal/mol) of conventional drugs of AD and flavonoids when docked against AChE.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Aricept (Drug)	-5.0
2.	Exelon (Drug)	-5.91

3.	Razadyne (Drug)	-5.0
4.	Catechin	-8.3
5.	Luteolin	-8.9
6.	Apigenin	-9.1
7.	Naringenin	-8.6
8.	Quercetin	-8.9
9.	Pelargonidin	-7.5

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against AChE, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of AChE. The binding of the ligands with AChE have been illustrated in figure 12.

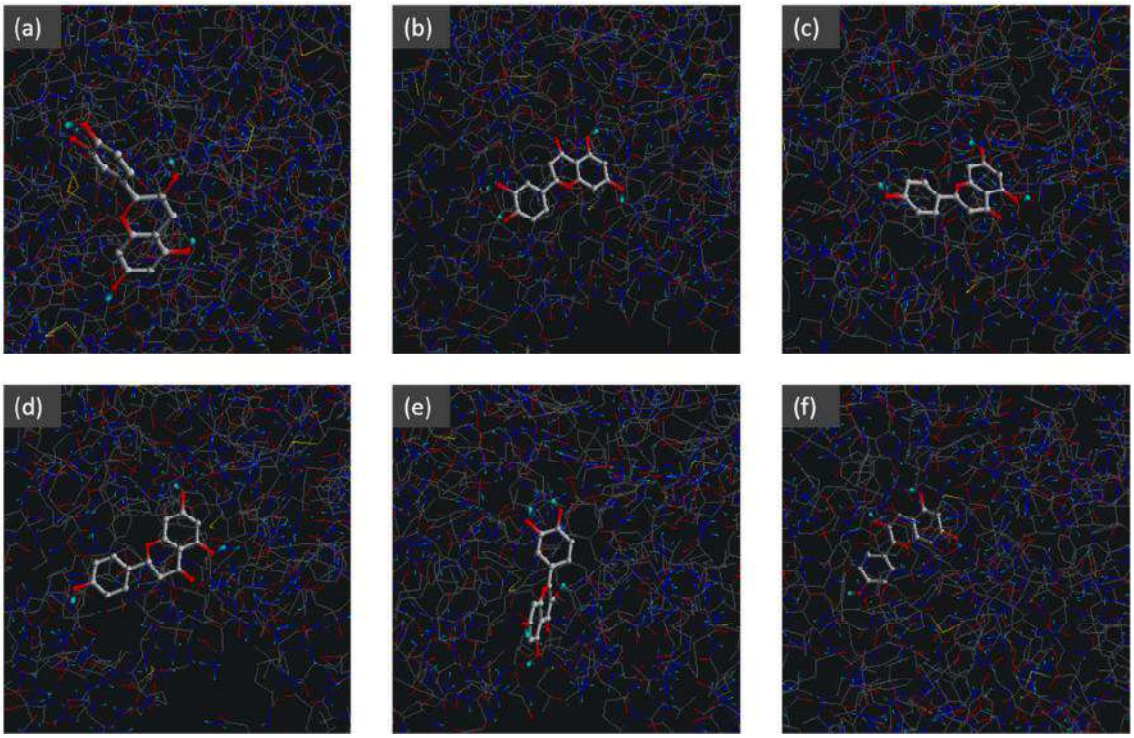

Figure 12: Docking study of Acetylcholinesterase (AChE) with the screened flavonoids. (a) AChE interaction with Catechin, (b)AChE interaction with Luteolin, (c) AChE interaction with Apigenin, (d) AChE interaction with Naringenin, (e) AChE interaction with Quercetin, and (f) AChE interaction with Pelargonidin.

4.8.2 Parkinson's Disease (PD)

The receptor chosen was dopamine. It was docked against the conventional drugs approved by FDA that are Levodopa, Apomorphine, Bromocriptine, Pramipexole, Ropinirole, Rotigotine,

and Carbidopa; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 10:

Table 10: Binding Affinity (kCal/mol) of conventional drugs of PD and flavonoids when docked against Dopamine.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Levodopa (Drug)	-6.3
2.	Apomorphine (Drug)	-7.6
3.	Bromocriptine (Drug)	-10.7
4.	Pramipexole (Drug)	-6.1
5.	Ropinirole (Drug)	-6.2
6.	Rotigotine (Drug)	-7.1
7.	Carbidopa (Drug)	-6.2
8.	Catechin	-7.3
9.	Luteolin	-8.3
10.	Apigenin	-8.1
11.	Naringenin	-8.1
12.	Quercetin	-8.6
13.	Pelargonidin	-7.0

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against Dopamine, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of Dopamine. The binding of the ligands with Dopamine has been illustrated in figure 13.

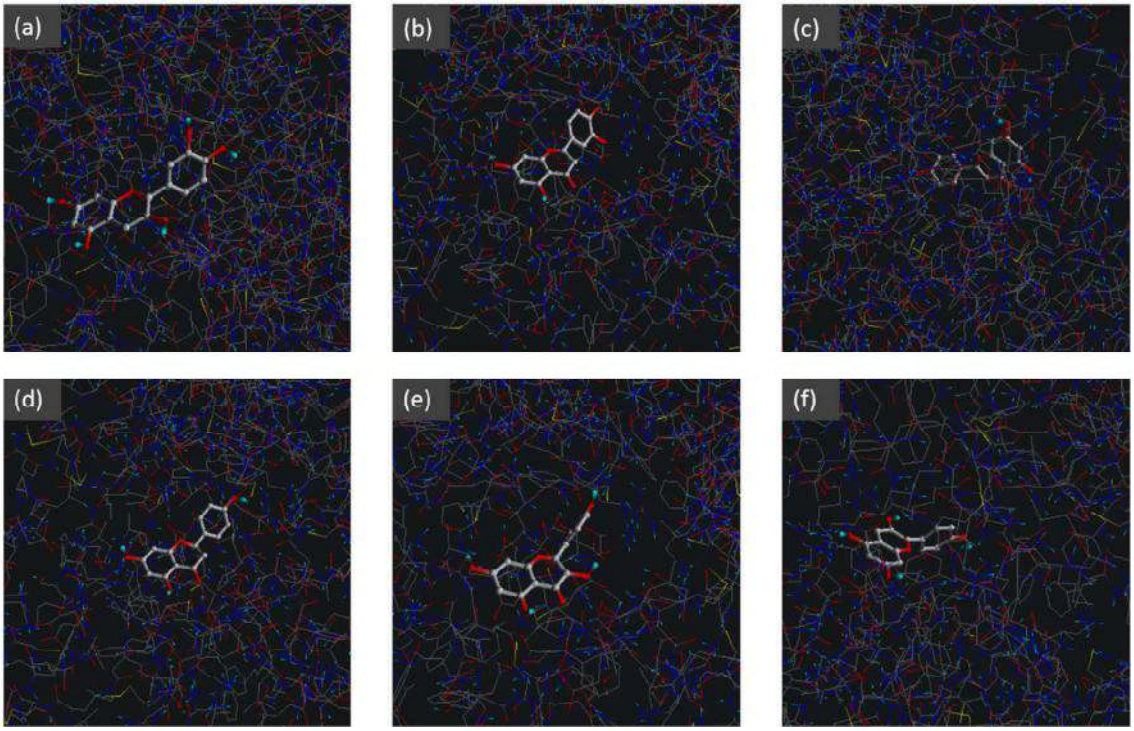

Figure 13: Docking study of Dopamine (DA) with the screened flavonoids. (a) DA interaction with Catechin, (b) DA interaction with Luteolin, (c) DA interaction with Apigenin, (d) DA interaction with Naringenin, (e) DA interaction with Quercetin, and (f) DA interaction with Pelargonidin.

4.8.3 Amyotrophic Lateral Sclerosis (ALS)

The receptor chosen was Soluble Carrier Family 22 Member 6 (S22A6). It was docked against the conventional drugs approved by FDA that are Riluzole and Edaravone; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 11:

Table 11: Binding Affinity (kCal/mol) of conventional drugs of ALS and flavonoids when docked against Soluble Carrier Family 22 Member 6.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Riluzole (Drug)	-6.6
2.	Edaravone (Drug)	-6.7
3.	Catechin	-8.3
4.	Luteolin	-9.2
5.	Apigenin	-8.6
6.	Naringenin	-8.2
7.	Quercetin	-8.5
8.	Pelargonidin	-8.2

From the above table, it is clearly visible that the flavonoids have binding affinities more than

the conventional FDA approved drugs when docked against S22A6, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of S22A6. The binding of the ligands with S22A6 has been illustrated in figure 14.

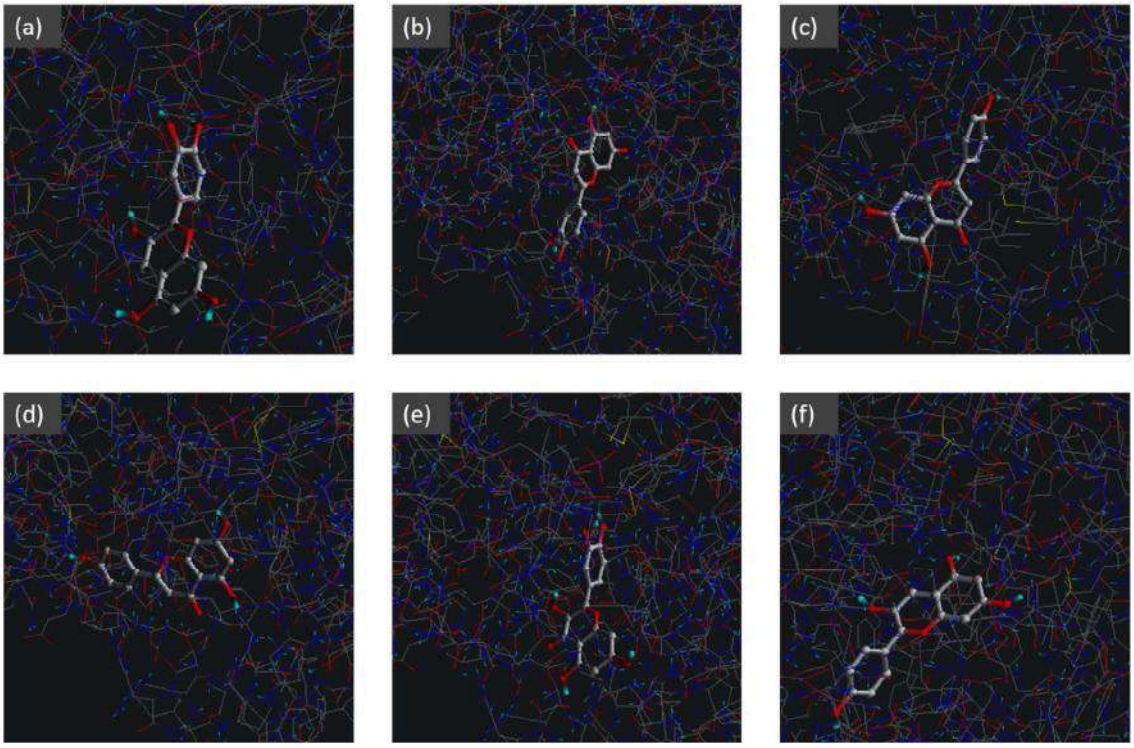

Figure 14: Docking study of Soluble Carrier Family 22 Member 6 (S22A6) with the screened flavonoids. (a) S22A6 interaction with Catechin, (b) S22A6 interaction with Luteolin, (c) S22A6 interaction with Apigenin, (d) S22A6 interaction with Naringenin, (e) S22A6 interaction with Quercetin, and (f) S22A6 interaction with Pelargonidin.

4.8.4 Huntington's Disease

The receptor chosen was Synaptic Vesicular Membrane Transporter (VAT-1). It was docked against the conventional drugs approved by FDA that are Tetrabenazine and Deutetrabenazine; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 12:

Table 12: Binding Affinity (kCal/mol) of conventional drugs of HD and flavonoids when docked against Synaptic Vesicular Membrane Transporter.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Tetradibenazine (Drug)	-6.5
2.	Deutetradibenazine (Drug)	-7.5

3.	Catechin	-8.0
4.	Luteolin	-8.7
5.	Apigenin	-8.4
6.	Naringenin	-9.4
7.	Quercetin	-8.2
8.	Pelargonidin	-7.2

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against VAT-1, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of VAT-1. The binding of the ligands with VAT-1 has been illustrated in figure 15.

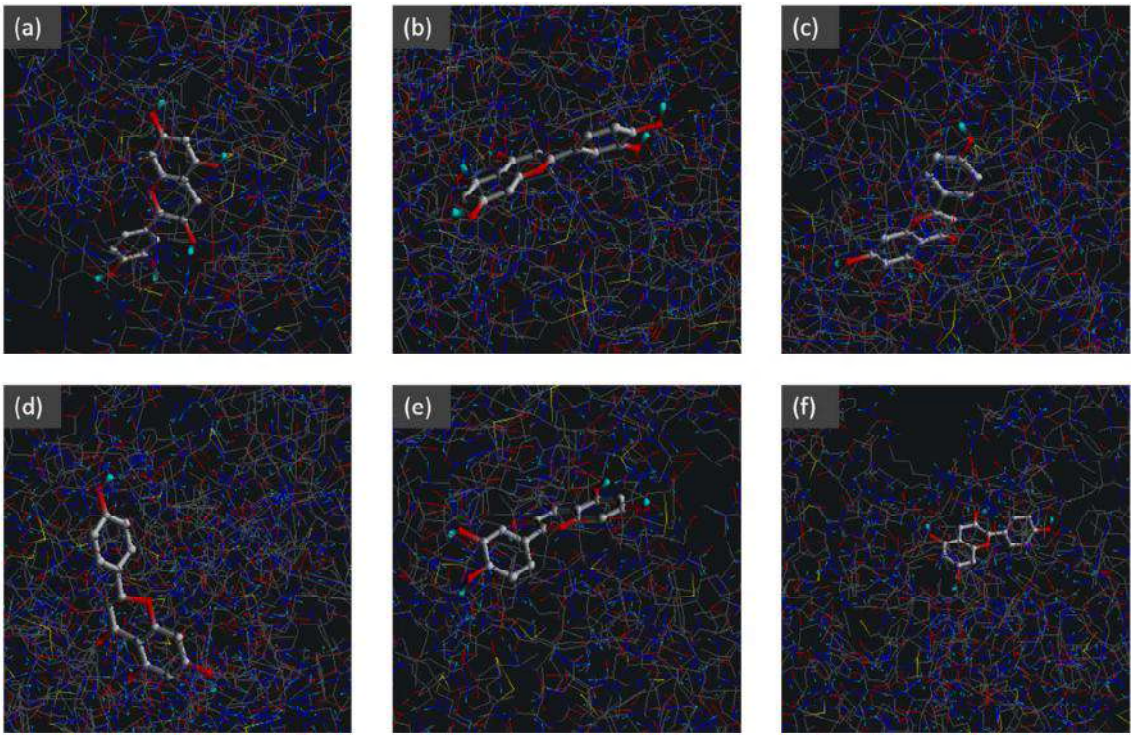

Figure 15: Docking study of Synaptic Vesicular Membrane Transporter (VAT-1) with the screened flavonoids. (a) VAT-1 interaction with Catechin, (b) VAT-1 interaction with Luteolin, (c) VAT-1 interaction with Apigenin, (d) VAT-1 interaction with Naringenin, (e) VAT-1 interaction with Quercetin, and (f) VAT-1 interaction with Pelargonidin.

From the above docking studies of the four diseases, it is clear that there are six flavonoids, namely, Catechin, Luteolin, Apigenin, Naringenin, Quercetin and Pealrginidin have shown a better binding affinity to the respective targets of the diseases, in comparison to the FDA approved drugs. All these flavonoids belong to different classes of flavonoids. Catechin and

Quercetin belong to flavonol class; Luteolin and Apigenin belong to the flavone class; Naringenin is a flavonone; and Pelargonidin is an anthocyanidin. All these can be obtained from a number of herbs and spices. Hence, these flavonoids can be used as a whole or in combination with other flavonoids to make a drug that can be used for the treatment of the NDDs.

4.9 Prediction of Bioactivity Score

The bioactivity score of the compounds was predicted using the Molinspiration Software.

Table 13: Bioactivity Score of the Flavonoids predicted using the Molinspiration Software.

S.No.	Flavonoid	GPCR Ligand (GPCR)	Ion Channel Modulator (ICM)	Kinase inhibitor (KI)	Nuclear Receptor Ligand (NRL)	Protease Inhibitor (PI)	Enzyme Inhibitor (EI)
1.	Catechin	0.41	0.14	0.09	0.60	0.26	0.47
2.	Luteolin	-0.02	-0.07	0.26	0.39	-0.22	0.28
3.	Apigenin	-0.07	-0.09	0.18	0.34	-0.25	0.26
4.	Naringenin	0.03	-0.20	-0.26	0.42	-0.12	0.21
5.	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
6.	Pelargonidin	-0.18	-0.11	-0.07	0.03	-0.33	-0.02

From table 13, it can be clearly seen that catechin has bioactivity score >0.0 for all the parameters, and hence it is highly active. Luteolin, apigenin, and quercetin have bioactivity score between 0.0 and -0.5, for the parameters, GPCR, ICM, and PI, hence show moderate activities against these parameters. But they show high activities against the parameters KI, NRL, and EI as their bioactivity score is more than 0.0. Naringenin shows moderate activities against ICM, KI, and PI (bioactivity score between 0.0 and -0.5) and high activity against the GPCR, NRL, and EI (bioactivity score >0.0). Pelargonidin is moderately active for all the parameters (bioactivity score between 0.0 and -0.5), except for NRL. Nuclear receptor ligands (NRLs) are important pharmaceutical targets due to their function as key regulators of many metabolic and inflammatory diseases, including diabetes, dyslipidemia, cirrhosis and fibrosis [89]. From the above table, it can be clearly seen that the flavonoids show high activity against NRL (bioactivity score >0.0).

4.10 In silico determination of antioxidant activity of the screened flavonoids

The antioxidant activity of the screened flavonoids was determined using the *in silico* analysis. The software used for the antioxidant analysis was the PASS online server. The parameters for the antioxidant activity of the screened flavonoids have been depicted in table 14.

Table 14: Antioxidant Activity of the screened flavonoids using PASS online.

S.No.	Flavonoids	Pa Antioxidant	Pa Free Radical Scavenger	Pa Anticarcinogenic
1.	Catechin	0.810	0.842	0.795
2.	Luteolin	0.775	0.755	<0.7
3.	Apigenin	0.732	0.719	<0.7
4.	Narigenin	0.794	0.769	0.724
5.	Quercetin	0.872	0.811	0.757
6.	Pelargonidin	NA	NA	NA

To use any compound as a drug, the compound has to fulfill certain conditions. From the above table, it can be seen that all the screen flavonoids have Pa values to act as an antioxidant more than 0.7, and hence they can be used as drugs (as whole or in combination with other flavonoids). The prediction could not be carried out for the Pelargonidin because it has a molecular charge of +1.

5. CONCLUSION

Flavonoids are secondary metabolites present in plants and hence have no or lesser. They are also present in the herbs and spices that we use in our day-to-day lives in our Indian households and hence are easily available for use, in comparison to the conventional drugs. We started with twenty-seven flavonoids and after predicting their molecular properties, drug-likeness properties, and pharmacokinetic properties we were left with six flavonoids. Their toxicity was also determined, in which the flavonoids proved to be less toxic in comparison to the FDA approved drugs as they were neither carcinogenic nor harmful to the liver in the long run. The LD50 values of the flavonoids were also less than the FDA approved drugs, proving them to be less toxic. After performing docking of these six flavonoids with the drug targets of the respective diseases, we came to a conclusion that all the screened flavonoids, Catechin, Luteolin, Apigenin, Naringenin, Quercetin, and Pelargonidin showed better binding affinities with the drug targets in comparison to the conventional drugs itself. To use these flavonoids in the form of drugs, they can either be used as a whole or in combination with other flavonoids. To know the efficacy of the flavonoids as a drug, their bioactive score was predicted, in which all the flavonoids were highly or moderately active. With the help of this *in* silico study, we could just find out how, herbs and spices that are present in Indian households can be proved effective to be used for the treatment of the NDDs.

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Neurodegenerative diseases

by Shruti Thareja

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ABSTRACT

Neurodegenerative diseases are still incurable and the current medications that are available can just control the symptoms of the diseases with a number of harmful side effects. In order to reduce these effects, natural compounds such as secondary metabolites can be thought of as a replacement. One of the categories of the secondary metabolites are the flavonoids that are known to possess a number of significant health benefits for the humans and thereby can as a replacement for the conventional drugs that are approved for the various NDDs because of their better effectiveness and fewer or no side effects. These flavonoids can be obtained from various natural sources such as the Plantae Kingdom. A part of the Plantae Kingdom are the herbs and the spices, which are used in food and beverages since time immemorial to enhance the color, flavor, and aroma. These herbs and spices are the easily available things in a household. For this, a database of the flavonoids that are present in the Indian herbs and spices was created and they formed the basis of this study. The study deals with the screening of the flavonoids by predicting their ADME predictions, toxicity, bioactivity, and docking. And thereby docking the screened flavonoids with the drug targets of the various NDDs, in order to limit the utilization of the conventional drugs. By this *in silico* study, we would be able to predict the pharmacokinetic properties and compare the binding affinities of the conventional drugs with the screened flavonoids.

Keywords: Drug-Likeness, Bioactivity, Flavonoids, *in silico*, Indian herbs and spices, Molecular Docking, Neurodegenerative Diseases, Pharmacokinetic Properties.

1. INTRODUCTION

Nature remains to be the most important source of medicines for the human welfare, since time illegitimate. The plant kingdom is a great source of bioactive compounds, which due to their intrinsic properties can be used for treating various diseases in humans related to the cardiovascular system, immune system, and nervous system. The plants in the recent times, have been the most common topic for scientific studies due to their medicinal properties and also because of the low toxicity, economic viability of the plants. Because of these properties, plants can be used a good source of phytopharmaceuticals. And, there is a global need to explore the plant kingdom in order to improve mental health, cognitive functions, and also to enhance the focus and concentration.

Nature is a repository of biological and chemical diversity [1]. Since many decades, it has been seen that the escalation of an immense number of scientific studies that have been focused on the activity of the non-nutritional compounds that are present in the diet and that are able to prevent the incidence of various degenerative diseases like cancer, cardiovascular diseases and neurodegenerative diseases [2]. Medicinal plants contain carious phytochemicals that are extractable and can be utilized for various scientific surveys. Many secondary metabolites are used in the pharmaceutical industry. In recent times, medicinal plants have gained a lot of importance due to their lesser side effects in comparison to the synthetic drugs and also due to the fact that they can meet the medicinal needs of the increasing human population.

The later part of human life is usually affected with one or the other neurological condition like loss of cognitive ability, loss of memory, etc. prolonged state of these symptoms leads to a condition called Dementia. Dementia is the first stage of any neurological disorder usually.

Neurodegenerative Disorder (Greek neuro – "nerval" and Latin degenerate, "to decline" or "to worsen"), is a heterogenous group of progressively deteriorating conditions that act on specific areas of the CNS and the PNS, thereby heading to gradual and progressive cognitive impairments, depending upon the type of the nerve cell that is undergoing degeneration in that particular disease [1]. The major characteristic features of the neurodegenerative disorders are ataxias (impairment in movement) and dementia (decline in memory) [3]. Some neurodegenerative diseases can be due to the genetic mutations and some are also related to the surrounding hazardous environmental conditions. Along with brain aging, there can be many other causes of neurodegeneration also like:

inflammation, oxidative stress, deposition of aggregated proteins, activation of various apoptotic factors. However, till today date, their main causes are still not known. The neurodegenerative disorders are:

1. Alzheimer's Disease (AD)
2. Parkinson's Disease (PD)
3. Huntington's Disease (HD)
4. Amyotrophic Lateral Sclerosis (ALS)
5. Multiple Sclerosis
6. Schizophrenia
7. Seizure disorders, etc.

Out of the above-mentioned disorders, the most common ones are the Alzheimer's Disease and the Parkinson's Disease. They are responsible primarily is affecting the life span and the life quality of the elderly [3].

Despite the progress in examining the pathology of the various neurodegenerative diseases, the cure is still unknown. Whatever drugs and treatments are known till today date, they are just capable of controlling the symptoms and are unable to completely cure the root cause of the disease and make the patient disease-free. This is because the disease is detected very late as the symptoms appear later than the onset of disease, so it is difficult to analyze whether the person is suffering from the disease or not.

Herbs and spices have been used in a multiple number of ways since time immemorial. Since the ancient times, herbs and spices are being added to the food to enhance flavor and also to improve the organoleptic properties [4]. As defined by the Food and Drug Administration (FDA), spices are: " aromatic vegetable substances, in the whole, broken, or the ground form, whose significant function is seasoning in food rather than nutrition" [5]. Spices have been used for purposes such as medicine, religious, cosmetic, vegetables or perfumery, other than flavoring [6]. Herbs and spices (especially in the dried form) contain a high content of polyphenols and other physiologically active phytochemicals [7].

Phytochemicals are a varied group of bioactive compounds that are present in the plants[8] and include flavonoids, alkaloids, terpenoids, phenols and lignans. Due to their wide range of chemical,

biochemical, and molecular characteristics, they can be of great interest for the neurodegenerative diseases[8]. Phytochemicals have proved to be promising candidates for various pathological conditions such as modulation of multiple signaling pathways and behaving as antioxidant and anti-inflammatory agents [9]. agents against cancer and various neurodegenerative diseases or in some cases as antifungal agents too [10]. Phytochemical rich diets are usually associated with increased longevity and a wide range of health benefits such as a decreased incidence of cardiovascular diseases and a slowed progression of cerebrovascular diseases [11]. The most common and abundant phytochemicals are the flavonoids.

Flavonoids are polyphenolic compounds. They are naturally occurring, biologically active compounds, that are abundantly found in fruits and vegetables [12]. Due to the variations in the C-ring in the structure of the flavonoids, they are classified as flavonols, flavones, isoflavones, flavanol, flavonones, and anthocyanins [13]. Flavonoids and their metabolites have great affects on the human and animal health, because of their antioxidant properties. Hence due to these antioxidant properties, flavonoids become the potential candidates for the study on neurodegenerative diseases. Flavonoids are also important for, the suppression of lipid peroxidation, modulation of gene expression, inhibition of inflammatory inhibitors. Flavonoids also help to maintain the endogenous antioxidant status of the neurons and thereby protecting them from any kind of degeneration [8]. They are the only class of secondary metabolites that can cross the blood brain barrier (BBB). Hence, their multiple effects have drawn the interest of the scientists towards flavonoids and discovering their role in neurodegenerative diseases.

The main objectives of this project are:

1. To create a database of the Indian spices and herbs along with their constituent flavonoids.
2. To find out the 3-D structure of the proteins, flavonoids and the conventional drugs.
3. To estimate the molecular and drug-likeness properties of the flavonoids.
4. To estimate the pharmacokinetic properties of the flavonoids.
5. To predict the toxicity of the screened flavonoids.
6. To predict the binding affinity between the proteins and the flavonoids and the conventional drugs and compare them.
7. To predict their bioactivity and antioxidant activity of the screened flavonoids.

2. REVIEW OF LITERATURE

2.1 NEURODEGENERATIVE DISEASES: AN OVERVIEW

The ongoing industrialization, changes in life style, and excessive use of herbicides and pesticides and other toxic chemicals that are used in the production of food materials are seriously threatening to the life of humans and are posing various health hazards. These toxic chemicals produce neurotoxins that affect the chemical transmission among the neurons and thereby causing neurodegenerative diseases [14].

Millions are affected yearly with neurodegenerative diseases worldwide. According to the World Health Organization (WHO), 737 million people were affected with neurodegenerative diseases worldwide in the year 2009, all of these people were of 60 years of age or more. This no is expected to increase to 2 Billion by the year of 2050.

Neurodegenerative diseases are a group of progressively deterioarating conditions that affect certain areas of the CNS and the PNS, leading to a gradual decrease in cognitive and movement impairments depending upon the type of the nerve cell that is undergoing degeneration [1]. There are various causes of these neurodegenerative diseases such as protein degradation [15], various environmental factors [16], familial history [18,19], mitochondrial defects [19], abnormal protein accumulation in neurons [20], etc. however, the most general cause of these diseases is aging [19].

There are many Neurodegenerative Diseases, some of them have been mentioned below:

- Alzheimer's Disease (AD)
- Parkinson's Disease (PD)
- Huntington's Disease (HD)
- Amyotrophic Lateral Sclerosis (ALS)
- Multiple Sclerosis
- Schizophrenia
- Seizure disorders, etc.

Alzheimer's Disease and the Parkinson's Disease are the most common NDDs. They occur in 40-60% of the patients [21].

The brain and spinal cord are made up of an innumerable different neurons that have different functions, such as controlling movements, making decisions and providing sensory information [1]. Since these neuronal cells cannot regenerate themselves, hence their degradation can be devastating. NDDs are thereby characterized by the gradual loss of the sensory and the motor neurons and also the loss of the ability of the mind to send sensory information to an external object [22]. Neurodegenerative diseases are divided into two different groups [1], namely:

1. Movement related problems, such as ataxia
2. Dementia and memory related problems.

The biological mechanisms that are associated with neurodegenerative diseases are aggregation of proteins in neurons, oxidative stress, abnormal ubiquitination, mitochondrial dysfunction, depletion or inadequate synthesis of neurotransmitters, excitotoxicity of neurons as well as disarrangement, degradation of neurotransmitters in the synaptic cleft due to the increased activity of enzymes or blood brain barrier (BBB) [23].

The most common form of the NDDs is Dementia that is affecting millions worldwide and continue to affect them as the age progresses. It is not a disease rather it is a group of symptoms, leading to various other diseases or conditions [1], being characterized by chronic progressive mental disorder, affecting the memory, comprehension, thinking, language and calculation [1].

2.1.1 Developmental Stages of Neurodegenerative Diseases

There are three developmental stages of neurodegenerative diseases. In this section, each stage has been described briefly along with the symptoms that appear at each stage.

- **Retrogenesis:** The beginning of the NDDs is the malfunctioning of the cholinergic system of the basal forebrain, which reaches the entorhinal cortex and hippocampus that are responsible for the short and long-term memory [24]. This results in the modifications in the brain which usually starts 10-20 years before and the first signs of NDD is forgetfulness and short term memory loss [25]. The disease with its further progression starts affecting the cerebral cortex. This stage is linked with the clinical diagnosis of NDDs in patients which include losing decision power, confusing in similar places, mood and personality changes, missing valuable things, increased anxiety, loss of spontaneity and sense of initiatives [24].

- **Cognitive Dysfunction:** There is a relation between neurodegeneration and toxic proteins. It is accompanied with the increase in pathological neurofibrillary plaques and tangles in the entorhinal cortex (EC), caudate, substantia nigra [24]. In order to keep the memory alive, the connection between EC and hippocampus has to be maintained, and any difficulty in these two regions can disrupt the circuit and lead towards memory damage and disorder.
- **Gait Abnormality:** Predicting a gait abnormality indicates a disturbance in the cognitive functions. A term has been proposed, " Last-In-First-Out", which refers to the phenomenon in which the neural circuits that mature late in the developmental life cycle are more vulnerable to damage, i.e., neurodegeneration [26]. Disturbances in cognitive function are directly linked with higher gait disturbances and is one of the major symptoms of the brain syndrome [27].

2.2 ALZHEIMER'S DISEASE

Alzheimer's Disease being the most common form of dementia and hence the neurodegenerative disorder which occurs among older people above the age of 60 years. Once, this disease was considered as a rare disease and now it has become the most common in almost every household and is affecting millions of people worldwide. It was discovered in 1906 by a German neuropathologist and psychiatrist named Alois Alzheimer and hence the disease is named after him.

AD is a type of dementia causing problems related to memory, thinking and behavior. Symptoms usually develop steadily and get worsen over the time, becoming sever enough that it starts interfering with the day-to-day tasks. AD accounts for 60 to 80% of the total cases of dementia worldwide. The greatest known risk factor of AD is age, and the majority of people with this disease are usually 65 years or older. AD gets worsen with time and age, since it is a progressive disorder. In the initial stages, patients just have mild memory loss but with gradual progression they start losing the ability to carry on a conversation and response to the environment.

2.2.1 Affects of Alzheimer's Disease on the Brain

There are complex changes that occur in the brain during the onset and the progression of the disease. The changes in the brain start occurring a decade before the onset of the actual cognitive symptoms. The early stages of the AD are usually symptom-free, but there are toxic changes that

are occurring in the brain. In AD, there are abnormal depositions of proteins from amyloid (Aβ) plaques and tau tangles (neurofibrillary tangles, NFT). Once, the health neurons stop functioning, they lose connections with the other neurons and therefore, they die. The damage initially appears in the hippocampus and the entorhinal complex (EC) of the brain, which are primarily responsible for the memories. As the disease progresses, more and more neurons die, affecting the specific brain area to shrink. By the time, the disease has reached the end of the first stage, the tissue of the brain has been reduced to a greater extent. The difference between a healthy and AD affected brain has been shown below in the figure 1.

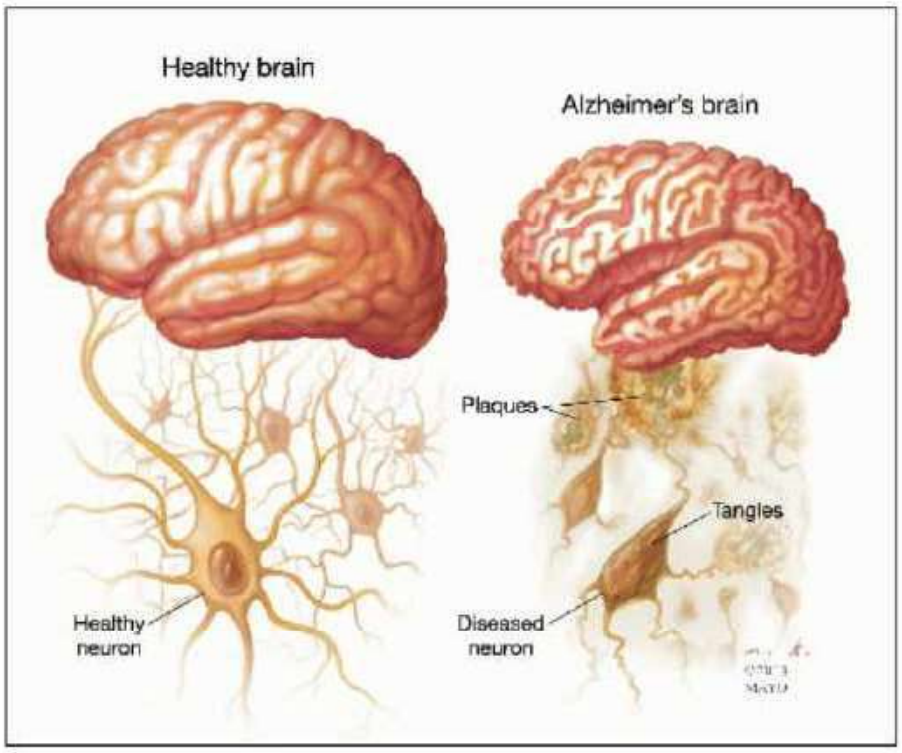

Figure 1: Difference between healthy brain and Alzheimer's brain. (Source: Mayo Clinic)

2.2.2 Causes of Alzheimer's Disease

Scientists feel that in people with early onset of AD, genetic mutations are usually the cause of the disease. And in those people with late onset of AD, there can be a complex series of brain changes that can occur over the time. These changes can be attributed to environmental, genetic, and lifestyle factors. Some of the main causes of AD have been described below:

- **Age-related changes in the brain:** As we all know that AD is an age-related disease that affects the people of age 65 and above. The main reason that it strikes in later stage of life is that, as the age of the person progresses, the brain also undergo certain changes. These changes are autrophy (shrinking) of certain parts of the brain, inflammation, production of free radicals (unstable molecules) and mitochondrial dysfunction (breakdown of energy production within the cell).
- **Genetics:** Another cause for AD is genetics. Due to genetics, one can say whether the disease was an early onset or late onset. A gene called apolipoprotein E (APOE) is involved in the late-onset of AD. This gene has got several forms. One of them is the APOE ε4, it increases the person's risk of developing the disease and is also associated with the development of early-onset of AD. However, carrying the gene APOE ε4 does not necessarily mean that the person will develop the disease, as people who didn't carried this gene had developed AD according to the research.

  Also, a number of regions in the genome have been identified by the researchers that might be responsible for causing the disease to varying degrees.

  Early-onset AD is generally caused in people between the age group of 30 to 60 and less than 5 % of the people develop the disease. Most of the cases are due to the changes in one of the three genes for AD, and this type of disease is known as the early-onset familial Alzheimer's Disease or FAD.

  Most of the people with Down's Syndrome can develop AD. This is because in case of Down's Syndrome, there is an extra copy of the chromosome 21 which is supposed to have the gene responsible for producing harmful amyloid.
- **Health, Environment and Lifestyle Factors:** Researchers suggest that beyond genetics, health, environment and lifestyle factors also play an important role in causing AD. A nutritious diet, physical activity, social engagement, and mentally stimulating pursuits have all been important in helping people to stay healthy as they age. These factors might also help to reduce the risk of cognitive decline and AD.

2.2.3 Types of Alzheimer's Disease

There are two types of AD:

- **Early-onset AD:** This type of AD is very rare, only 10% of the population have this type of AD. It usually occurs among the people between the age group of 30 and 60. Some cases are due to an inherited change in one of the three genes.

  The three single-gene mutations that are associated with the early-onset of AD are:

  - Amyloid Precursor Protein (APP) on chromosome 21
  - Presenilin 1 (PSEN1) on chromosome 14
  - Presenilin 2 (PSEN2) on chromosome 1

  Gene mutations result in the production of abnormal proteins that are associated with the disease and play an important role in breaking down the APP. This breakdown leads to the generation of Amyloid Plaques, a hallmark of AD.

- **Late-onset AD:** Maximum cases of Ad are of this type. People with the late-onset of AD start developing symptoms somewhere around the age of 60. Till today date, no specific gene responsible for causing AD has been identified. But the presence of apolipoprotein E (APOE) on the chromosome 19 does increase the risk of AD. The APOE is responsible for carrying out cholesterol and other types of fats in the bloodstream.

  There are three different forms of the APOE. They are:

  - APOE ε2 is quite rare and might also help in providing protection against the disease. If AD occur in some person having this allele, it would occur too late in the person.
  - APOE ε3 is the most common allele. It is believed to play a neutral role, i.e., neither decreasing nor increasing the risk of the disease.
  - APOE ε4 increases the risk of AD and is also associated with the early-onset of AD. Having one or two alleles of APOE ε4, increases the risk of the development of the disease. About 25% of the people carry one allele and almost 2 to 3% people carry two alleles. This gene increases the risk of development of the disease in the person. But it is not necessary that if the person carries the allele, he/she will develop a disease. In some cases, it has been seen that people who didn't carried the allele also developed the disease and those carrying the allele didn't developed the disease.

2.2.4 Signs and Symptoms of Alzheimer's Disease

An inability to retain the very recent events is the first symptom of AD. With the progression in the disease, impairment in different areas of cognition (for instance, language, abstract reasoning, and executive function or decision making), start occurring to unpredictable degrees and it archetypally coincides with difficulty occurring at work or in any social condition or various household activities [28]. Mood changes also accompany the deteriorating memory[29]. Delusions and psychotic behavior are not naturally triggering signs but can occur at any time during the course of the disease[30]. The occurrence of psychosis during the initial stages of dementia suggests some other diagnosis like presence of dementia with Lewy Bodies [28].

A family history of dementia is considered to be on the most important risk factors for AD [31]. About half of such cases result due to the mutations in the genes encoding APP, presenilin 1 or presenilin 2 [32]. The disease is also more concordant among monozygotic twins as compared to the dizygotic twins [33].

As the disease progresses, the conditions start getting worse and the following symptoms start appearing:

- Confusion, disorientation, and getting lost in familiar places
- Difficulty in planning or making decisions
- Problems with speech and language
- Personality changes, such as becoming aggressive, demanding and suspicious of others
- Problems in moving around without any assistance or performing self-care tasks
- Hallucinations or delusions
- Low mood or anxiety

2.2.5 Stages of Alzheimer's Disease

There are basically four stages of the AD. The disease along with their characteristics have been discussed below in the following table:

Table 1: Changes that occur at various stages during progression of AD.

	Mild	Moderate	Severe
Activities of daily living (ADL)	Problems with the routine tasks	Needs help with basic ADL (e.g., feeding, dressing, bathing)	Progresses to total dependence on the caregiver

Behavior	Changes in personality and mathematical changes	Anxiety, suspicion, pacing, insomnia, agitation, wandering	Crying, screaming, groaning
Cognition	Confusions and memory loss, e.g. misplacing objects, forgetting names, disorientation	Difficulty in recognizing family and friends and chronic loss of memory	Loss of speech and misidentifies or is unable to recognize familiar people

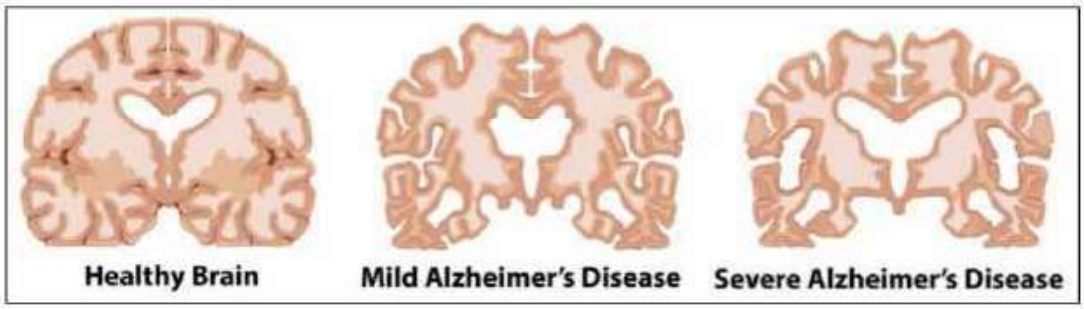

Figure 2: Progression of the Alzheimer's Disease (Source: National Institute on Aging)

2.2.6 Treatment of Alzheimer's Disease

A number of medicines have been prescribed for the treatment of AD. The various medicines are:

- Acetylcholinesterase (AChE) inhibitors

  AChE is an enzyme participating in the cholinergic neurotransmission. It breaks down acetycholine into acetate ion and choline, which terminates the neurotransmission process [34]. The role of AChE inhibitors is to enhance the level of acetycholine in the brain [1]. Hence AChE inhibitors play an important role by inhibiting the AChE from breaking down the acetycholine and thereby continuing the neurotransmission process.

  Donepezil, galantamine, and rivastigmine are the medicines that are usually prescribed by the doctors for the people with early to mild AD. People with severe AD are also advised to take these medications, but in many cases it has not proved to be effective.

  There are various side effects of these drugs like nausea, loss of appetite, and vomiting.

- Memantine

  This medicine is not an AChE inhibitor, it works by blocking the effect of an excessive amount of the chemical called gumtamate in the brain.

  Memantine is used for moderate or severe AD. It is usually prescribed for those patients that cannot take or are unable to tolerate the AChE inhibitors.

It can be taken along with the AChE inhibitors also. It can cause certain side effects too. Side effects include headaches, dizziness and constipation, but these are usually temporary.

2.3 PARKINSON'S DISEASE (PD)

Globally, the second most common NDD is the Parkinson's Disease. More than 10 million people are affected with PD worldwide and it account for 2% of the population, older than 65 years of age. It was first described as a neurological syndrome by James Parkinson in the year 1817, hence the name of the disease.

PD is caused by the deficit of the dopaminergic neurons in the substantia nigra (SN) and striatum parts of the brain which further leads to a decrease in the dopamine (DA) level [35]. The loss of DA level to the striatum and the SN leads to imbalance with neurotransmitters such as AChE and DA, resulting in PD symptoms [36]. The symptoms of the disease, gradually worsen over the time and age since it is progressive disorder. It leads to difficulty in walking and talking as the disease progresses. Both men and women can be affected with PD, but 50% more men are affected than by women.

2.3.1 Effect of Parkinson's Disease on the Brain

The property that makes it different from other movement disorders is that the cell loss occurs only in a specific region of the brain, i.e., the SN. The nerve cells in this region appear dark or black when seen under the microscope, as the Latin for SN is a "black substance".

The neurons in the SN, produce a neurotransmitter called the dopamine. Dopamine helps in the regulation of movement. Thereby, categorizing it as a movement disorder. In addition to the loss of dopamine, the protein α-synuclein are also affected in the disease. This protein tends to form aggregates that are called Lewy Bodies in the patients that are affected with PD.

As the disease progresses, more and more neurons die in the SN region of the brain, as can be seen in the figure 3. There is a very small proportion of the people getting affected by the disease which

is an early onset of the disease. The early onset of the disease usually starts at the age of 40 years in some cases and in some can start after 50 years of age.

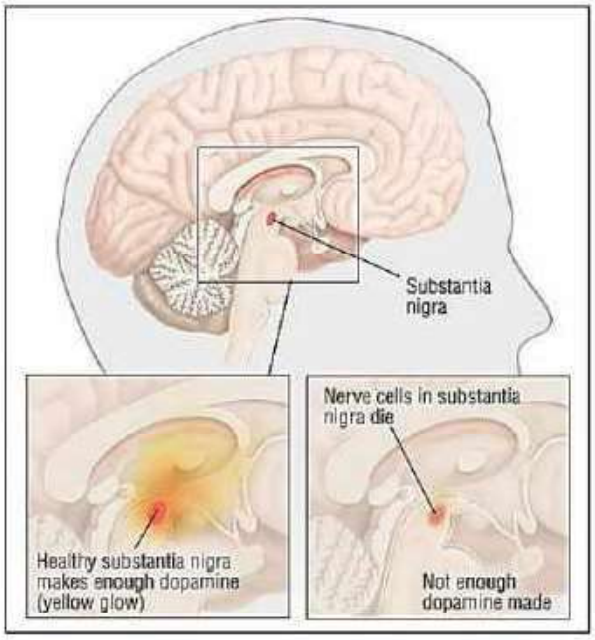

Figure 3: Difference between healthy brain and Parkinson's Disease brain. (Source: Harvard Medical School)

2.3.2 Causes of Parkinson's Disease

The cause of Parkinson's Disease is still unknown. It can be possible that there can be more than one cause of the disease. Scientists believe that both the environmental factors and genetics play an important role to cause the disease. Some of the factors have been described below:

- **Genetics:** A number of genetic factors have shown to increase the risk of the disease in a person. The pathway how the genetic factors increase the susceptibility of the disease are still unknown. It can run in families, being passed on from one generation to another, but such cases are rare.

  The most common genetic effect that triggers the disease in a person is the mutation in the gene LRRK2. Mutations in the protein α-synuclein are also known to trigger the disease.
- **Environmental Factors:** Certain environmental factors such as exposure to pesticides or herbicides that are used in the farming and traffic or industrial pollution may trigger the condition. Traumatic brain injury, known to alter the levels of consciousness also increases

the risk of the disease. Exposure to heavy metals can be thought of a as a contributor in the onset of the disease.

- **Other causes:** "Parkinsonism" is the term that is used to describe the symptoms like tremors, muscle rigidity, and slowness of movement. Parkinson's Disease is the most common type of parkinsonism. There can be a multiple cause included under parkinsonism such as antipsychotic medications that can affect the brain.

2.3.3 Types of Parkinson's Disease

There are following types of Parkinson's Disease:

- **Primary Parkinsonism or Idiopathic PD:** It is the most common type of PD. The term idiopathic signifies that the cause is unknown. The most common symptoms of this are tremor, rigidity, and slowness of movement.
- **Secondary Parkinsonism:** It is also called Parkinsonism Syndrome or Atypical Parkinsonism or Parkinson's Plus. In this condition, the cause of the disease is known, but it is difficult to differentiate it from the primary parkinsonism except the fact that it doesn't respond to the dopaminergic medications like levopoda. Secondary parkinsonism is of the following types:
  - **Multiple System Atrophy (MSA):** This is the advance form of the idiopathic PD. In this, there is an overproduction of the protein α-synuclein. This results in damaging major parts of the brain. It progresses at a faster rate than the idiopathic PD. Its average onset is in the mid-50's. It is difficult to distinguish it from normal PD, other than the fact that it doesn't respond to the PD medications. Its symptoms include ataxia and dysfunction in the automatic nervous system.
  - **Drug-induced Parkinsonism:** This condition is caused as a result of the side effects of the medications especially those affecting brain dopamine levels. This is the most common form of the secondary parkinsonism. It is difficult to differentiate it from the idiopathic PD.
  - **Vascular Parkinsonism (VP):** It can also be considered as a cerebrovascular disease. This type of parkinsonism is caused by a series of small strokes that can cause in the death of certain areas of the brain. VP usually affects the lower body

and memory loss. VP doesn't respond to the normal PD medications. It becomes more common with age, especially with the people who are affected with diabetes.

- **Normal Pressure Hydrocephalus (NPH):** This condition is similar to VP. This condition can be treated by removing the spinal fluid as a short-term treatment and for the long-term treatment, lumbar puncture can be done to permanently divert the spinal fluid.
- **Corticobasal Degeneration (CBD):** It is the least common type of Parkinsonism. It is caused due to the build-up of the protein tau, which damage various parts of the brain. It usually starts by affecting limb on one side of the body and then slowly spread to other parts with time. It typically begins after the age of 60 and progresses more rapidly than the PD.
- **Progressive Supranuclear Palsy (PSP):** It is the most common type of secondary parkinsonism. The cause of PSP is similar to that of CBD, i.e., the build-up of the protein tau, leading to damage in various parts of the brain. Those with PSP often have problem in swallowing, difficulty in producing speech, memory loss and sleeping problems.

2.3.4 Signs and Symptoms of Parkinson's Disease

PD has the following main symptoms:

- Trembling in hands, leg, jaw, or head.
- Stiffness in the limbs and the trunk.
- Bradykinesia (slowness of movement)
- Rigidity (muscle stiffness)
- Impaired balance and coordination

Other symptoms may include depression, problems in swallowing, chewing, speaking problems, skin problems, sleep disturbances. The main symptom is memory loss. It is difficult to test the onset of the disease.

Symptoms and the rate of progression of the disease vary from person to person. The symptoms can be physical or cognitive.

Physical symptoms of PD include: balancing problems, anosmia (loss of smell), nerve pain, constipation, dizziness, blurred vision or fainting, hyperhidrosis (excess of sweating), insomnia, drooling.

Cognitive and Psychiatric symptoms include: depression, anxiety, dementia which may include hallucinations in some people, memory problems, problems in decision making and organization.

People with PD usually develop a parkinsonian gait which makes them lean forward and reduce swinging of arms. They may also have trouble in continuing movement. Symptoms usually occur on one side of the disease and then spread to the rest of the body.

2.3.5 Treatment of Parkinson's Disease

There is currently no treatment available for the disease. The treatment that is available is to control the symptoms of the disease and to lead a quality life. These treatments include:

- Supportive therapies
- Medications
- Surgery

Supportive therapies: It includes physical, occupational and speech therapies. Physical therapy can improve the gait developed due to the disease and can help in improving the right exercise regimen. It can also help the patient get some relief from the muscle stiffness and the joint pain. Occupational therapies are used to improve the motor skills. Speech therapies are used to improve the speech and the language barriers developed due to the progression of the disease. It can also help to get rid of the swallowing and the chewing problems developed due to the disease.

Medications: Medicines that are prescribed for PD include: drugs for increasing the dopamine levels and the drugs that can help in controlling the nonmotor symptoms.

The most common medicine that is used for treating PD is levodopa, L-dopa. L-dopa helps to replenish the levels of dopamine in the brain. To minimize the side effects of L-dopa, it is usually taken with carbidopa. There can be many other medications to treat the disease like: dopamine agonists to mimic the role of dopamine in the brain, MAO-B inhibitors, COMT inhibitors, and anticholinergic drugs.

Surgery: surgery is considered in those cases where patients have stopped reacting to the medications. The surgery which is most common and has been approved by the FDA is Deep Brain Stimulation (DBS). It involves implanting an electrode into the parts of the brain such as subthalamic nucleus (STN) or the globus pallidus interna (GPI). This electrode acts similar to the pacemaker. The electrode is connected to a pulse generator which runs through the wires and it stimulates the part of the brain that is affected by the disease. It helps to stop movement related problems of the disease like rigidity, slowness of movement, and tremor.

2.4 AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ALS or Amyotrophic Lateral Sclerosis is a fatal NDD along with AD and PD. It affects the brain and the spinal cord. It is an idiopathic NDD of the human brain [35]. ALS was first described by Charcot in 1874. It is also known as Lou Gehrig's Disease, after the name of it's first patient Lou Gehrig. It is a progressive disorder that involves damage to the motor neurons at different levels [37]. Motor neurons are the ones that run from the brain to the spinal cord and from the spinal cord to various parts of the body. The difference between a healthy neuron and the one affected with ALS has been depicted in the figure 4.

"Amyotrophic" refers to the muscular atrophy, weakness and fasciculation that implies it to be the disease of the lower motor neurons [38]. "Lateral Sclerosis" refers to the hardness of the anterior and the lateral columns of the spinal cord [38]. ALS occurs between the age of 40 and 70 years. Usually the most common age for the occurrence of the disease is 55 years.

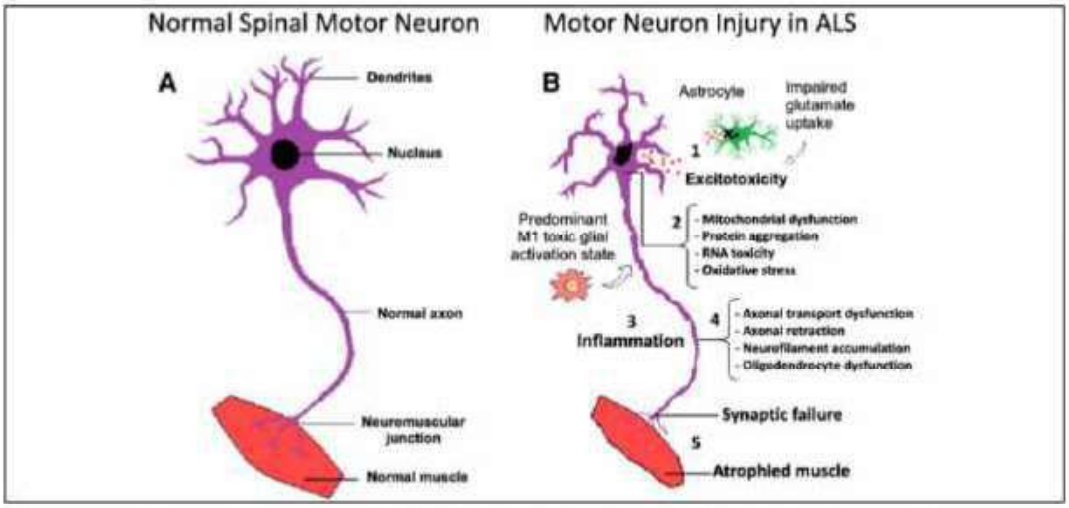

Figure 4: Difference between a healthy neuron and ALS affected neuron [39].

2.4.1 Causes of Amyotrophic Lateral Sclerosis

The causes of ALS are not known. But the studies done by the scientists suggest that both the genetic as well as the environmental factors play a role in the development of the disease.

- **Genetics:** In 1993, scientists discovered that the gene *SOD1* was associated with the familial type of ALS. Other genes that can cause the disease are *ALS2, ALS4, VAPB, angiogenin*. Certain changes in the processing of the RNA can also lead to the degeneration of the motor neurons, thereby causing the disease. RNA molecules in the body are involved in the synthesis of the proteins and regulating the gene expression and activity, the mutations in the structure and shape of the motor neurons can also play a role in causing the disease.
- **Environmental Factors:** an increased susceptibility to the environment toxins can cause the disease. Unhealthy diet, physical trauma, behavioral and occupational factors can also lead to the disease.

2.4.2 Types of Amyotrophic Lateral Sclerosis (ALS)

There are basically two types of ALS:

- **Sporadic ALS:** Majority of the ALS cases are sporadic. In this type, the disease suddenly occurs at random with no associated environmental risk factor or genetic factors. The

family members of the patient with sporadic ALS are at a greater risk of developing the disease.

- **Familia (Genetic) ALS:** about 5 to 10% of the total cases are familial. It is caused, if one of the parents carries the gene responsible for causing the disease. A number of genes have been identifying to cause the disease. But mainly the mutations in the genes *SOD1* and *C90RF2* are known to cause the disease in most of the cases.

2.4.3 Signs and Symptoms of Amyotrophic Lateral Sclerosis

Both the sporadic and the familial ALS are associated with the progressive loss of the motor neurons. But the symptoms depend upon to which part of the brain is being affected. Some of the early symptoms are:

- Fasciculations in the arm, shoulder, leg or tongue
- Muscle cramps
- Tight and stiff muscles
- Muscle weakness
- Slurred and nasal speech
- Difficulty in chewing or swallowing
- Tripping or falling
- Persistent fatigue

As the disease progresses, it can lead to paralysis in certain cases. Symptoms can first appear either in the hands or the legs, calling it the "limb onset" ALS or problems in swallowing or speaking, calling it the "bulbar onset" ALS. Regardless of where the symptoms appear first, muscle weakness and atrophy continue to get worsen as the disease progresses. People with ALS can face difficulty in breathing as the muscles of the respiratory system get weakened. They eventually lose the ability to breath on their own and hence they depend on the ventilator. Affected individuals also have a greater risk of pneumonia at the later stages.

2.4.4 Treatment of Amyotrophic Lateral Sclerosis

As the disease progresses, it becomes more and more difficult for the patient to breath and digest food. Till today date as such there is no cure for the diseases. The medications and the therapies that are suggested by the medical professionals can only reduce the symptoms to lead an easy life.

- **Medications:** Till today date, Riluzole and Edaravone are the two drugs that have been approved by the FDA to be used for the treatment of the disease. Riluzole reduces the damage done to the motor neurons by decreasing the levels of glutamate, which is a transporter between the nerve cells and the motor neurons. Edaravone reduces the clinical assessment in daily functioning with the people affected by ALS.

  The physicians can also prescribe medications to reduce muscle cramps, depression and the other symptoms related to the disease.
- **Physical Therapy:** It can help the patient to strengthen muscles, improve cardiovascular health, and help fight fatigue and depression. Physical Therapy involves exercises such as walking, swimming, and stationary bicycling.
- **Speech Therapy:** It helps the patients to improve their speaking ability and benefits them by improving their communication ability which had been affected due to the disease. Computer aids such as computer-based speech synthesizers can also help them.

2.5 HUNTINGTON'S DISEASE (HD)

Huntington's Disease is a complex NDD that affects the CNS [40]. It is genetic NDD that is passed down from the parents to the child. It caused due to one single defective gene on chromosome 4. This defect is dominant, hence would be transferred over the generations. The disorder was first discovered by George Huntington in the 1800s.

The defective gene causes the build-up of the protein called Huntington, that damages the nerve cells in the brain thereby causing the disease. During the course of this disease, there is a gradual development of involuntary muscle movements affecting the hands, face, feet, trunk and cognitive impairment.

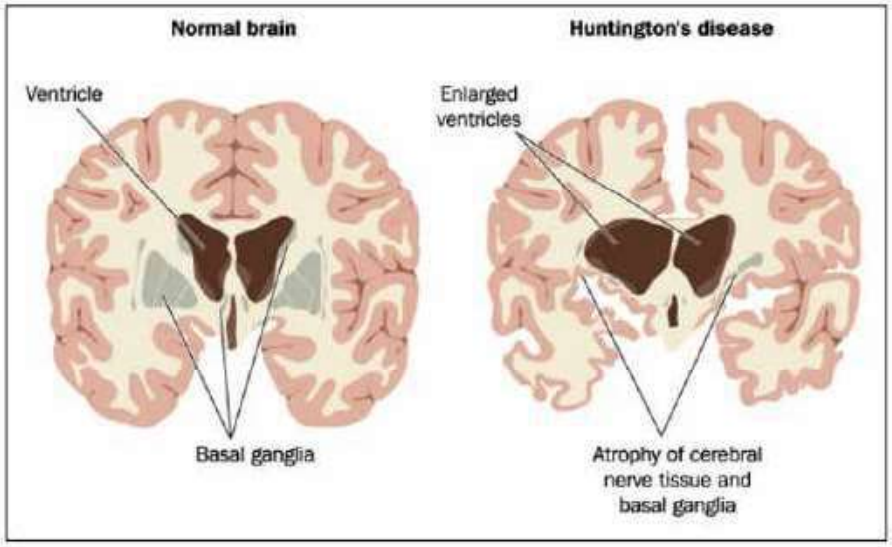

Figure 5: Difference between a healthy brain and HD brain. (Source: Medanta)

2.5.1 Causes of Huntington's Disease

HD is an autosomal dominant inherited disorder. It is caused by mutations of the gene located on the short arm of the chromosome 4. This gene provides instructions for making up a protein called huntington. The function of this protein is still unknown, but it does play an important role in neurons in the brain.

In this mutation, the gene has a DNA segment known as CAG trinucleotide repeat. This segment is made up of three building blocks namely, cytosine, adenine and guanine. Usually, a healthy person has 10 to 35 copies of the CAG segment. But in a HD affected individual, the number of copies of this segment is repeated 36 to 120 times, which leads to an overproduction and an abnormally long segment of the huntington protein. The elongated protein is cut into smaller pieces and forms aggregates in the neurons of the brain. the following image shows how an elongated protein is created (Figure 6).

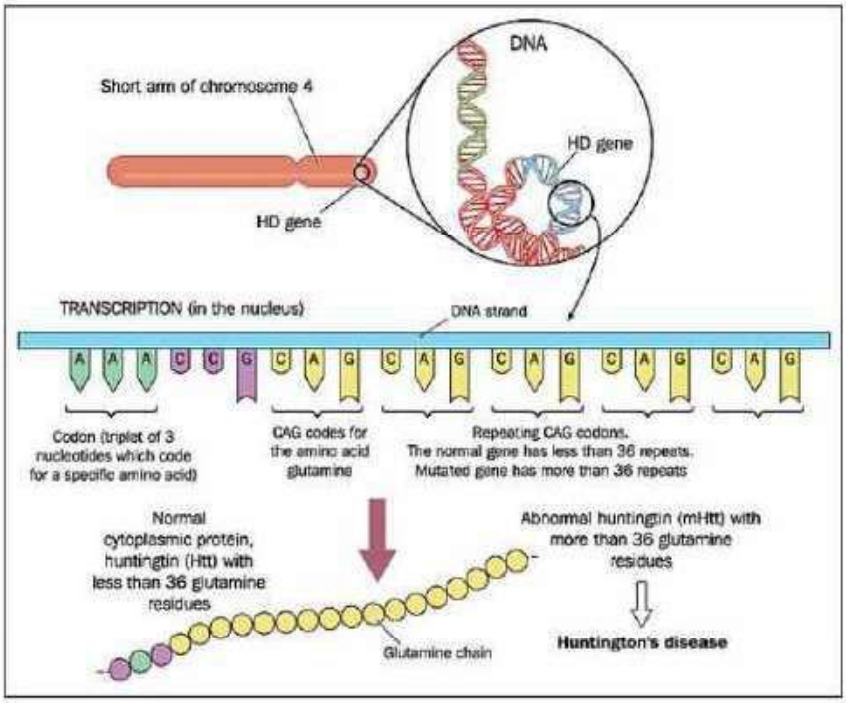

Figure 6: Process of creating an elongated protein. (Source: NIH)

2.5.2 Types of Huntington's Disease

HD is of the following two types:

- **Adult-onset HD:** This condition is the most common. It occurs in people who are in their thirties or forties. Early symptoms can include irritability, depression, small involuntary movements, poor coordination, and trouble making new decisions and learning new things. Individuals with the adult-onset of the disease live for up to 15 to 20 years after the signs and symptoms begin to appear.
- **Juvenile-onset HD:** This condition either begins in the childhood or adolescence. It involves movement problems, mental and emotional changes. Additional symptoms of this condition can be drooling, clumsiness, frequent falling, and slurred speech. Seizures occur in 30 to 50% of the children. Their school performance drops. This condition progresses more rapidly than the adult-onset condition, and the patients can live up to 10 to 20 years after the signs and symptoms start appearing.

2.5.3 Signs and Symptoms of Huntington's Disease

The symptoms can start appearing in the 30s or 50s, and in some cases, it can be as early as 2 years of age or as late as 80 years of age. It is characterized by rapid uncontrollable movements such as tics or muscle jerks. It also causes a decline in thinking and reasoning skills, including memory, concentration, and the ability to plan and organize.

The brain changes can lead to depression, sudden mood swings, irritability and uncharacteristic anger. The most common symptom is that the person develops obsessive-compulsive disorder, asking the same questions over and over again. Patients with the disease are at a greater risk of developing pneumonia due to being bedridden all the time and poorly nourished.

2.5.4 Treatment of Huntington's Disease

There is currently no cure for HD. The treatments that are available they focus on managing symptoms.

In August 2008, FDA approved the drug Tetrabenazine for the treatment of involuntary movements associated with HD. This is the only drug that has been approved for the disease.

Other treatments are providing therapies. Occupational therapy to help make everyday tasks easier. Speech and language therapy for the communication and feeding problems. Physiotherapy to help the individual with movement and balance.

2.6 INDIAN SPICES AND HERBS

Spices are the dried, pleasantly aromatic parts of the plant. As defined by the Food and Drug Administration (FDA), spices are: " aromatic vegetable substances, in the whole, broken, or the ground form, whose significant function is seasoning in food rather than nutrition" [5]. Spices are substances that have been used since time immemorial as a food additive for the purpose of flavoring and adding colors, and sometimes as preservatives to kill the harmful bacteria [41]. Spices have been used for purposes such as medicine, religious, cosmetic, vegetables or perfumery, other than flavoring [6]. The main difference between a herb and a spice is that spices are obtained

from all the parts of the plants other than the leaves, whereas the herbs can be obtained only from the leaves [42].

Most of the herbs and the spices originate from the Mediterranean countries, especially the Middle East or Asia, and they have been used since ancient times in the day-to-day lives, specifically in the Indian kitchens [43]. Herbs and spices have played, and still continue to play, important roles as flavoring agents, food preservatives and medicines. Over the past few de- cades, a lot of research into their health benefits has increased significantly, due to their ability to treat various chronic diseases [5]. Their potential health benefits include conferring protection for cancer, neurodegenerative diseases, cardiovascular diseases, obesity, and type 2 diabetes [44], [45], [46], [47], [48].

The positive health effects of herbs and spices is towards preventing the chronic diseases appear to be due to the direct action of their constituent phytochemicals (particularly polyphenols) targeting specific receptors or enzymes involved in various anti-inflammatory pathways or immune responses [49]. Herbs and spices (especially in the dried form) contain a high content of polyphenols and other physiologically active phytochemicals [7].

2.7 SECONDARY METABOLITES

The sum of all the biochemical processes that take place in an organism is called metabolism. The main primary metabolites of plants are produced through the fundamental metabolic pathways such as Glycolysis, Calvin Cycle, Krebs Cycle, etc. The main primary metabolites are the carbohydrates, lipids, proteins, and nucleic acids. They are found in plants and their main function is to help the plants to survive [50].

Plant secondary metabolites are considered to have irrelevant fundamental role in preserving the life processes in plants, but they do play a significant role in the interaction of the plant with its surrounding environment for adaptation and defense.

Secondary metabolites, also known as natural products, that are the products of the metabolism process not essential for normal growth, development or reproduction of an organism, whereas they are necessary for meeting the requirements of the organisms. They help the organisms in surviving the interspecies competition, providing defensive mechanisms and also facilitating the reproductive process. They also accord to the specific odors, colors and tastes in plants [51].

Secondary Metabolites are inimitable sources for pharmaceuticals, food additives, flavors, and industrially important pharmaceuticals [52] and they also have significant practical applications in medicinal, nutritive, and cosmetic purposes besides playing a role in plant stress physiology for adaptation [53]. Over 2,14,000 secondary metabolites are known till now, and they have been classified according to their rich diversity, structure, function, and biosynthesis. Secondary Metabolites are basically categorized into the following classes:

1. Terpenoids and steroids
2. Fatty acid-derived substances and polykitedes
3. Alkaloids
4. Nonribosomal polypeptides
5. Enzyme cofactors

2.7.1 Role of Secondary Metabolites

Among the Plantae Kingdom, the angiosperms are spread over the majority of the terrestrial surface, contributing exceptionally to the biomass in terms of their volume and weight in compared to the other life forms combined together [54].

Plants have to overcome a number of challenges, like seed dispersal, fluctuations in the supply of the basic nutrients for their survival and their interdependency with the other organisms and herbivores in their proximate environment. Hence, during the course of evolution, the plants have developed numerous secondary biochemical pathways allowing them to synthesize innumerable chemicals while responding to their specific environmental stimuli [54][55]. They do have any substantial role in the primary metabolic requirements of the plants, rather they increase the overall surviving ability of the plants and in overcoming the challenges when interacting with their immediate environment [56].

There are various other roles of secondary metabolites such as they act as a general host for various protective roles thereby helping the plants to defend itself from the microorganisms. They also help the plant to manage the inter-plant relationships [57]. Here, it is clear that the primary role of the secondary metabolites is to act as a feeding deterrence, and because of this these phytochemicals can be toxic to the potential herbivores, by interacting directly with the CNS and

the PNS of the herbivore [58]. In this regard, secondary metabolites act as antagonists or agonists of neurotransmitter systems [59][60] or form structural analogs of the endogenous hormones [61].

In order to survive, plants also have to foster innumerable symbiotic relationships. Another role of the secondary metabolites lies here that is attracting the pollinators by the various colors and scents. Thereby providing an attractive chemical for the predator resulting in the synthesis and release of a cocktail of phytochemicals to attract the natural predators of the herbivore [57].

2.8 FLAVONOIDS

In recent times, there has been an interest in the potential role of the flavonoids to modulate neuronal function and prevent age-related neurodegeneration. Flavonoids refer to a class of secondary metabolites that have a polyphenolic structure. They are the most abundant in fruits, vegetables and plant tissues [62] and thus they are included in the diet of the humans [63]. One of the most remarkable properties of flavonoids is that they have good antioxidant potential [64]. Thus, they have pharmacological importance as therapeutic agents. The physiological activity of the flavonoids have been reported to be due to their structure and geometry [65].

Based on their chemical structure they have been divided into flavonols, flavanols, flavones, flavanones, isoflavones, anthocyanidins, and chalcones. Till date, 9000 flavonoids have been reported, all present in fruits and vegetables. All the flavonoids share a common structure in the sense that they have two aromatic rings (A and B) which are connected by three carbon atoms, thereby forming an oxygenated heterocycle (ring C) (Figure 7). The variations in their saturation of the basic flavan system, their alkylation, and/or glycosylation and the hydroxylation pattern of the molecules form the basis of their subcategories that have been discussed above.

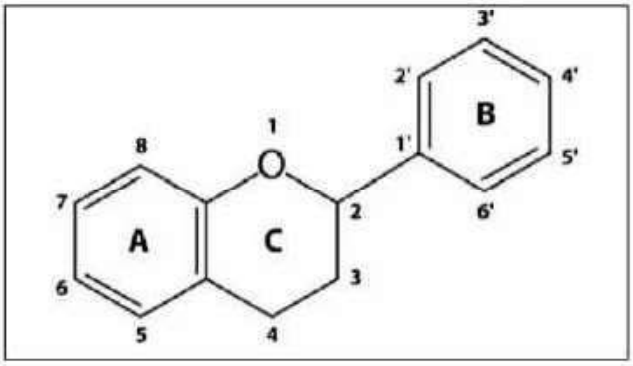

Figure 7: Basic Flavonoid Structure [66]

2.8.1 Properties of Flavonoids

Flavonoids possess a number of properties that attribute to their use in the treatment of various diseases. Some of the properties are described below:

1. They possess numerous biological effects such as antiviral, anti-inflammatory, anti-tumor, anti-allergic, antiplatelet, and antioxidant activities [67].
2. They can easily cross the Blood Brain Barrier (BBB) and may show neuropharmacological activities at the molecular level thereby manipulating the protein function and gene expression [13].
3. The performance of the spatial memory improves due to the improvement in the brain derived neurotrophic factor (BNDF) which is affected due to the dietary intake of the flavonoids [68].
4. Flavonoids can regulate the immune system of brain, and thus attenuate the neuroinflammation by inhibiting the production of nitric oxide and cytokines that are produced by the activated microglia [69].
5. They also possess the ability to modulate NFκB and mitogen-activated protein kinases (MAPKs) signaling pathways and also attenuate the function of COX-2, IL-6, IL-1β, and TNF-α [70].

The above properties of the flavonoids show that they can be a good fit for the NDD profile, and in a process dependent on the suppression of the lipid peroxidation, inhibition of inflammatory mediators, modulation of gene expression, and activation of antioxidant enzymes, they can protect the neurons from degenerating and thereby preventing the neurodegeneration [71], [72].

2.8.2 Classes and Sources of Flavonoids

Due to the multiple effects of the flavonoids, scientists have been drawn towards the investigation of the neuroprotective role of the flavonoids. The various classes and their sources of the flavonoids have been discussed in the Table 2.

Table 2: Classes and Sources of Flavonoids

Classes	Flavonoids	Common Sources
Flavonols	Rutin, Quercetin, Kaempherol, Myricetin	Leeks, onions, kale, broccoli, apples, cheeries, berries, red wine, tea
Flavanols	Catechin, Epicatechin, Epigallocatechin, Epigallocatechin gallate (EGCG)	Green tea, black tea, blueberries, tea, cocoa, grapes, chocolate
Isoflavones	Genistein, Daidzein, Glycetin, Formanantine	Legumes, soy beans, soy products,
Flavones	Luteolin, Apigenin, Acacetin,	Parsley, celery, apple skins, chrysanthemum flowers, cabbage, peppers, carrot
Flavonones	Hesperetin, Naringin, Isoxanthohumol, Taxifolin	Citrus fruits, tomatoes, grapefruits
Anthocyanidins	Cyanidin, Malvidin, Pelargonidin, Delphinidin	Red wine, berry fruits, cherries, grapes, kidney beans

2.8.3 Flavonoids: Treatment in Neurodegeneration

NDDs are characterized by the changes in the structural and the pathological conditions, thus innumerable targets and methods with better efficacy are needed for their treatment. Flavonoids affect the cell system by modulating the activity of the various metabolic pathways and thereby reducing the cognitive decline and the neuronal dysfunction [73] . They can also prevent or delay the onset of the NDD at their desired doses and concentrations.

3. METHODOLOGY

Virtual screening of the pharmacophore properties was done of the compounds, their structures were obtained. The ligand structures were obtained in the ".sdf" format and the protein structures were obtained in the ".pdb" format. Further, their docking was carried out against the well-known target of the diseases.

3.1 Software's and Databases

To conduct the study the following Databases were used to obtain the data:

- PubChem (https://pubchem.ncbi.nlm.nih.gov/) [74]
- ChEBI (https://www.ebi.ac.uk/chebi/) [75]
- DrugBank (https://www.drugbank.ca/) [76]
- RCSB PDB (https://www.rcsb.org/) [77]
- FooDB (https://foodb.ca/)

The software's that were used to predict the results are:

- Molsoft L.L.C.: Drug-Likeness and Molecular Property Prediction (https://molsoft.com/mprop/mprop.cgi)
- Molinspiration software (https://www.molinspiration.com/cgi-bin/properties)
- pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]
- PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]
- Open Babel [80]
- CASTp (Computed Atlas of Surface Topography of Proteins) (http://sts.bioe.uic.edu/castp/index.html?1ycs) [81]
- AutoDock Vina in PyRx [82]

3.2 Collection of data of Indian spices and their flavonoids

The list of the Indian Spices was created using the literature survey and their phytochemical constituents were summarized using several publications [83], [84], [85].

3.3 Selective prediction of the Molecular Properties

The molecular properties of the flavonoids were obtained using the software Molinspiration software (https://www.molinspiration.com/cgi-bin/properties). In this software, SMILES were used as the input format. The SMILES of the compounds were obtained from PubChem/ChEBI database. The RO5 is an important criteria to predict that the compound can be used as a potential drug in which the log P should not exceed 5, the molecular weight should not be more than 500 daltons, the number of hydrogen bond acceptors (HBA) should be less than 10 and the hydrogen bond donors (HBD) to be less than 5. Those flavonoids that violated the rule were omitted from the further analysis.

3.4 Selective prediction of the Drug Likeness Properties

The Drug Likeness properties were obtained using the software Molsoft L.L.C.: Drug Likeness and Molecular Property Prediction (https://molsoft.com/mprop/mprop.cgi). The SMILES were used as the input, which were obtained from the PubChem/ChEBI database. The value of the drug-likeness score has to be between 0 and 1. Those flavonoids that had the drug-likeness score less than 0 were not used for the further analysis.

3.5 Selective Prediction of the BBB value

The BBB (Blood Brain Barrier) is an important factor for a compound to be used as a drug as it must cross the BBB readily. The BBB value was calculated using the PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]. The criteria to differentiate the compounds was whether they had high, medium or low permeability. If the

- logBB>0.3, high permeability;
- logBB lies between 0.3 and -1.0, medium permeability;
- logBB<-1.0, low permeability.

Those flavonoids that showed high permeability were used for the further analysis.

3.6 Predicting the Pharmacokinetic Properties

The pharmacokinetic profile of a compound describes its ADME (absorption, distribution, metabolism, excretion and toxicity) properties. The prediction of the ADME properties was done in order to increase the success rate of the compound as a drug. The ADME properties were calculated for the flavonoids. The properties include Aqueous solubility, Blood Brain Barrier level, CYP 2D6, and Plasma Proetin Binding Level. The properties were predicted using the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78], in which the SMILES were used as an input obtained from the PubChem/ChEBI database.

Analysis of pharmacokinetic profiles of the ligands is an imperative assortment before heading on towards the preclinical and the clinical assessments. They help us to pinpoint whether the ligands have the potential to act as druggable molecules for the treatment of the various disorders. The outcomes define the conspicuous functionalities of the structures of the ligands [86].

Generally, only the unbound drug is available for diffusion or transport across cell membranes, and also for interaction with a pharmacological target. As a result, a degree of plasma protein binding of a drug influences not only on the drug's action but also its disposition and efficacy. If the Plasma Protein Binding (%PPB),

- PPB more than 90%, chemical strongly bound
- PPB less than 90%. Chemical weakly bound

3.7 Toxicity Determination of the Compounds

The toxicity of the compounds were determined with the help of the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]. The toxicity of the FDA approved drugs of the respective diseases were compared with the toxicity of the screened flavonoids. The properties include rat LD50, AMES toxicity, Maximum Tolerated Dose, and Hepatotoxicity.

The toxic potency of a compound is an important parameter, hence for this purpose lethal dosage (LD50) is a standard measurement. The LD50 is the amount of a compound given all at once that causes the death of the 50% of the group of test animals.

The AMES test is used to assess the compound's mutagenic potential using bacteria. If a compound is tested positive, that means it is mutagenic having the carcinogenic potential.

The maximum recommended tolerated dose (MRTD) provides an estimate of the toxic dose threshold of the chemicals in humans. If MRTD $\leq$ 0.477 log (mg/kg/day), it is considered low and if MRTD > 0.477 log (mg/kg/day), it is considered high.

Drug-induced liver injury is one of the major concerns for developing a drug. With the help of hepatotoxicity, one can predict whether a compound is affecting the normal functioning of the liver or not.

3.8 Analysis of the 3-D Structures of the target Proteins of the disease

The target proteins that were chosen were Acetylcholinesterase (AChE, PDB ID: 4PQE) for AD, Dopamine (DA, PDB ID: 6VMS) for PD, Synaptic Vesicular Membrane Transport (VAT-1, PDB ID: 6K9V) for HD, and Soluble Carrier Family 22 Member 6 (S22A6, PDB ID: 2KBI) for ALS. Only these target proteins were chosen because they were the targets for the FDA approved drugs and hence useful for the study in order to compare the inhibitory activities of the flavonoids against the similar drug targets. The 3-D structure of the target proteins were obtained from the RCSB PDB (Protein DataBank, https://www.rcsb.org/) [77] in the PDB format. These proteins would be acting as receptors for the docking analysis.

3.9 Identification of the 3-D Structures of the Ligands

The ligands that were chosen were of two types, conventional drugs approved by the FDA for the treatment of the various NDDs and the screened flavonoids.

(a) The 3-D structure of these drugs were obtained from the DrugBank (https://www.drugbank.ca/) [76]. The structure of the drugs was obtained in the SDF format and then were converted into the PDB format using Open Babel [80].

(b) The 3-D structure of the screened flavonoids was obtained from the PubChem Databse (https://pubchem.ncbi.nlm.nih.gov/) [74] and ChEBI (Chemical Entities of Biological Interest, https://www.ebi.ac.uk/chebi/) [75]. The structures were obtained in the SDF format and then were converted into the PDB format using the software Open Babel [80].

3.10 Analysis using Molecular Docking

Molecular Docking is a striking scaffold to understand the various drug bio-interactions [87]. It is the mechanistic study where a ligand is placed into the preferred binding site of the receptor to form a stable complex in a non-covalent fashion [87]. The docking analysis was performed using the AutoDock Vina in PyRx [82].

3.11 Bioactivity Prediction of the Flavonoids

The flavonoids that showed higher binding affinity than the FDA approved drugs with their target proteins were broke down with the help of the Molinspiration software (https://www.molinspiration.com/cgi-bin/properties) to check whether the compounds were bioactive or not.

The bioactivity scores include the activity of the compounds towards GPCR ligands, particle channel modulators, kinase inhibitors, molecular receptor ligands, protease inhibitors and other enzyme targets.

The compounds are said to be

- bioactive, if bioactivity score >0;
- moderately active, if bioactivity score lies between -5.0 to 0;
- and inactive, if the bioactivity score <-5.0.

3.12 Prediction of the Antioxidant Property of the Flavonoids

The flavonoids that showed the low binding affinity with the proteins were checked for their antioxidant properties using the PASS Server (http://www.pharmaexpert.ru/passonline/) [88]. The chemical structure in PASS is described by the descriptors called Multilevel Neighbourhoods of

Atoms (MNA). For each prediction, two probabilities, Pa and Pi are calculated based on the statistics of the MNA descriptors. Pa and Pi values range between 0.000 to 1.000 thereby indicating whether the compound is active or inactive, respectively [89]. The input for the PASS server were the SDF files of each of the flavonoids. It predicted the antioxidant activity in general and also the other activities that were related to it. Pa value was chosen as more than 0.7 (cut-off value) [90].

4. RESULTS AND DISCUSSION

4.1 Database of the Indian Spices and their flavonoids

The Indian spices that were chosen are the ones that have been used routinely. The database (table 3) of the Indian spices and their chemical constituents (flavonoids) was created with the help of several publications. A total of 24 Indian spices were taken into consideration.

Table 3: Database of the Indian Spices and their chemical constituents (flavonoids).

S.No.	Scientific Name	Common Name	Flavonoids Present
1.	*Curcuma longa*	Turmeric (Haldi)	Curcumin
2.	*Thymus vulgaris*	Thyme (Ajwain)	Apigenin, Chrysin, Luteolin, Diosmetin
3.	*Coriandrum sativum*	Coriander (Dhania)	Acacetin, Quercetin, Kaempferol
4.	*Ocimum basilicum*	Basil (Tulsi)	Quercetin, Catechin, Kaempferol, Rutin, Luteolin
5.	*Allium sativum*	Garlic (Lehsun)	Kaempferol, Myricetin
6.	*Nigella sativa*	Nigella (Kalonji)	Quercetin, Kaempferol
7.	*Azadirachta indica*	Neem	Quercetin, Kaempferol, Melicitrin
8.	*Sesamum indicum*	Sesame (Til)	Kaempferol, Naringenin, Apigenin, Quercetin, Myricetin, Luteolin, Rutin, Epicatechin
9.	*Myristica fragrans*	Nutmeg (Jaiphal)	Myricetin
10.	*Zingiber officinale*	Ginger (Adrak)	Quercetin
11.	*Syzygium aromaticum*	Cloves (Laung)	Kaempferol, Luteolin
12.	*Elletaria cardamomum*	Cardamom (Elaichi)	Quercetin, Kaempferol, Luteolin
13.	*Capsicum annuum*	Chilli Pepper (Capsicum)	Quercetin, Kaempferol, Luteolin, Catechin, Epicatechin, Rutin, Myricetin, Apgenin
14.	*Cinnamonum verum*	Cinnamon (Dalchini)	Gossypin, Gnaphalin, Hesperidin, Hibifolin, Hypolaetin, Quercetin
15.	*Trigonella foenum-graecum*	Fenugreek (Methi)	Vitexin, Tricin, Naringenin, Quercetin
16.	*Solanum lycopersicum*	Tomato	Naringenin, Rutin
17.	*Allium cepa*	Onion	Quercetin, Kaempferol, Isorhamnetin, Myricetin, Cyanidin
18.	*Laurus nobilis*	Bay Leaf (Tejpatta)	Kaempferol, Quercetin, Apigenin, Luteolin, Isorhamnetin
19.	*Cuminum cyminum*	Cumin	Kaempferol, Quercetin
20.	*Syzygium aromaticum*	Cloves	Kaempferol, Quercetin
21.	*Brassica*	Mustard	Isorhamnetin, Kaempferol, Quercetin
22.	*Crocus sativus*	Saffron	Quercetin, Kaempferol, Myricetin, Naringenin, Taxifolin, Tamarixetin, Isorhamnetin
23.	*Foeniculum vulgare*	Fennel seeds	Rutin, Quercetin, Kaempferol, Eriodictyol

24.	*Magnifera indica*	Amchoor (Dry mango powder)	Epicatechin, Catechin, Apigenin, Luteolin, Quercetin, Kaempferol, Myricetin

4.2 Prediction of Molecular Properties

A total of 27 flavonoids were obtained from the above created database. The molecular properties of the flavonoids were obtained using the Molinspiration software (https://www.molinspiration.com/cgi-bin/properties). For calculating these properties, the canonical SMILES of the flavonoids were obtained from the PubChem/ChEBI database and they were checked for the Lipinski's Rule of Five (RO5). The results are depicted in the table 4.

Table 4: Molecular Properties of the Flavonoids obtained using the Molinspiration Software.

S.No.	Flavonoid	miLogP	TPSA	n atoms	HBA	HBD	*n* rotb	MW	Volume
1.	Catechin	1.47	110.38	21	6	5	1	290.27	261.53
2.	Epicatechin	1.37	110.38	21	6	5	1	290.27	261.13
3.	Luteolin	1.86	111.13	21	6	4	1	286.24	272.86
4.	Apigenin	1.89	90.90	20	5	3	1	270.24	260.14
5.	Tangeretin	3.71	76.36	27	7	0	6	372.37	385.27
6.	Naringenin	1.75	86.99	20	1	5	3	272.25	251.12
7.	Myricetin	1.08	151.59	23	8	6	1	318.24	292.39
8.	Isorhamnetin	2.35	120.36	23	7	4	2	316.26	301.71
9.	Chrysin	2.94	70.67	19	4	2	1	254.24	216.03
10.	Rutin	-1.06	269.43	43	16	10	6	610.52	496.07
11.	Acacetin	3.00	79.90	21	5	2	2	284.27	241.58
12.	Diosmetin	2.28	100.13	22	6	3	2	300.27	249.59
13.	Kaempferol	1.70	111.13	21	6	4	1	286.24	268.99
14.	Gossypin	-0.62	230.73	34	13	9	4	480.38	380.22
15.	Quercetin	1.63	131.36	22	7	5	1	302.24	240.08
16.	Vitexin	1.63	181.05	31	10	7	3	432.28	391.88
17.	Gnaphalin	1.57	89.27	26	6	1	2	360.41	320.37
18.	Hesperidin	-0.55	234.30	43	15	8	7	610.57	511.79
19.	Hibifolin	-0.75	247.80	35	14	9	4	494.36	382.40
20.	Hypolaetin	1.71	131.35	22	7	5	1	302.24	240.08
21.	Tricin	2.30	109.36	24	7	3	3	330.29	275.14
22.	Taxifolin	0.71	127.44	22	7	5	1	304.25	246.32
23.	Tamarixetin	1.99	120.36	23	7	4	2	316.26	257.61

24.	Curcumin	2.30	93.07	27	6	2	8	368.38	332.18
25.	Eriodictyol	1.63	107.22	21	6	4	1	288.25	238.28
26.	Pelargonidin	-0.26	92.08	20	5	4	1	271.25	226.79
27.	Cyanidin	-0.75	112.31	21	6	5	1	287.25	234.81

The following violated one or more RO5: Myrictein had HBD >5; Rutin had HBA >10, HBD >5 and the TPSA >200; Gossypin had HBA >10, HBD >5 and the TPSA >200; Hesperidin had HBA >10, HBD >5 and the TPSA >200; Vitexin had HBD >5; and Hibifolin had HBA >10, HBD >5 and the TPSA >200

If any of the RO5 is violated that means that compound is not suitable to be used in the form of a drug. Excluding these 6 flavonoids, the rest 21 were then used for the further analysis.

4.3 Drug-Likeness Score Prediction

The Drug Likeness score was predicted using the Molsoft L.L.C.: Drug-Likeness and molecular property prediction (https://molsoft.com/mprop/mprop.cgi). The value of the drug-likeness score usually lies between 0 and 1. The drug-likeness score of the flavonoids have been depicted in the table 5.

Table 5: Drug-Likeness Score of the Flavonoids obtained using Molsoft L.L.C.: Drug-likeness and molecular property prediction software.

S.No.	Flavonoid	Drug-Likeness Score
1.	Catechin	0.64
2.	Epicatechin	0.64
3.	Luteolin	0.38
4.	Apigenin	0.39
5.	Tangeretin	-0.56
6.	Naringenin	0.82
7.	Isorhamnetin	0.39
8.	Chrysin	0.29
9.	Acacetin	0.29
10.	Diosmetin	0.06
11.	Kaempferol	0.50
12.	Quercetin	0.52
13.	Gnaphalin	-0.19

14.	Hypolaetin	0.25
15.	Tricin	-0.08
16.	Taxifolin	1.00
17.	Tamarixetin	0.16
18.	Curcumin	-0.82
19.	Eriodictyol	0.96
20.	Pelargonidin	0.51
21.	Cyanidin	-0.58

From the table, it is clearly visible that Tangeretin, Gnaphalin, Tricin, Curcumin, and Cyanidin have the negative drug-likeness score, hence they cannot be used in the form of a drug. Now, we have only 16 flavonoids available that would be used for further analysis.

4.4 Selective Prediction of BBB Value

The BBB permeability value of the 16 flavonoids was predicted using the PreADMET server (https://preadmet.bmdrc.kr/adme/) [79]. The criteria to differentiate the compounds was whether they had high, medium or low permeability. The predicted values of the screened flavonoids have been depicted in the table 6.

Table 6: BBB Permeability Value of the Flavonoids obtained using PreADMET Server.

S.No.	Flavonoid	logBBB Value	Permeability (High/Medium/Low)
1.	Catechin	0.39413	High
2.	Epicatechin	0.394913	High
3.	Luteolin	0.367582	High
4.	Apigenin	0.565113	High
5.	Naringenin	0.59697	High
6.	Isorhamnetin	0.0580929	Medium
7.	Chrysin	0.93256	High
8.	Acacetin	0.150309	Medium
9.	Diosmetin	0.201086	Medium
10.	Kaempferol	0.286076	Medium
11.	Quercetin	0.372765	High
12.	Hypolaetin	0.211296	Medium
13.	Taxifolin	0.166964	Medium
14.	Tamarixetin	0.127547	Medium

15.	Eriodictyol	0.380271	High
16.	Pelargonidin	0.53039	High

Only those flavonoids that showed logBBB value higher than 0.3 were chosen for further analysis, as according to the criteria mentioned in the materials and methodology, they were readily (high) able to cross the blood brain barrier. The flavonoids that were readily permeable were, Catechin, Epicatechin, Luteolin, Apigenin, Naringenin, Chrysin, Quercetin, Eriodictyol, and Pealrgonidin (total of 9 flavonoids for further analysis).

4.5 Pharmacokinetic Properties Prediction of the Screened Flavonoids

The 9 flavonoids that were obtained after the screening procedure, were then checked for their pharmacokinetic properties. The pharmacokinetic properties were obtained using the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) [78]. The properties described are Water solubility, CYP 2D6 (acts as a inhibitor or non-inhibitor), and Plasma Protein Binding efficacy. The pharmacokinetic properties of the screened flavonoids have been depicted in the table 7.

Table 7: Pharmacokinetic Properties of the Flavonoids obtained using the pkCSM Server.

S.No.	Flavonoid	Water solubility (log mol/L)	CYP 2D6 (Yes/No)	% PPB
1.	Catechin	-3.036	No	100.000000
2.	Epicatechin	-3.101	No	100.000000
3.	Luteolin	-3.02	No	99.717233
4.	Apigenin	-2.989	No	97.253409
5.	Naringenin	-3.24	No	100.000000
6.	Chrysin	-3.022	No	95.095683
7.	Quercetin	-3.085	No	93.236103
8.	Eriodictyol	-3.505	No	100.000000
9.	Pelargonidin	-3.399	No	100.000000

These results show that the flavonoids possess good pharmacokinetic properties and also, they satisfy all the parameters to be taken over as a good drug. Hence, these flavonoids can be used for the molecular docking analysis.

4.6 Toxicity Determination of the Bioactive Compounds

The toxicity of the screened flavonoids was determined using the pkCSM online tool. With the help of this server their LD50 (lethal dosage 50), AMES toxicity, and Hepatotoxicity values were determined.

The determined values of the various toxicity tests for the FDA approved drugs are shown in the table 8A, and for the flavonoids are shown in the table 8B.

Table 8A: Determined values of the toxicity tests of the FDA approved drugs.

S.No.	Drug	Disease	LD50 value	AMES Test	Hepatotoxicity
1.	Aricept	AD	2.999	No	No
2.	Exelon	AD	2.893	No	No
3.	Razadyne	AD	3.402	No	No
4.	Levodopa	PD	2.027	No	Yes
5.	Apomorphine	PD	2.419	Yes	No
6.	Bromocriptine	PD	3.268	No	No
7.	Pramipexole	PD	2.482	Yes	No
8.	Ropinirole	PD	2.573	No	No
9.	Rotigotine	PD	2.718	No	Yes
10.	Carbidopa	PD	2.417	Yes	Yes
11.	Tetrabenazine	HD	2.572	No	Yes
12.	Deutetrabenazine	HD	2.482	Yes	No
13.	Riluzole	ALS	2.443	No	Yes
14.	Edaravone	ALS	2.219	No	No

Table 8B: Determined values of the toxicity tests of the screened flavonoids.

S.No.	Flavonoid	LD50 value	AMES Test	MRTD	Hepatotoxicity
1.	Catechin	2.513	No	0.506	No
2.	Epicatechin	2.057	Yes	0.41	No
3.	Luteolin	2.285	No	0.872	No
4.	Apigenin	2.327	No	0.713	No
5.	Naringenin	2.132	No	0.372	No
6.	Chrysin	2.486	Yes	0.183	No
7.	Quercetin	2.449	No	0.956	No
8.	Eriodictyol	1.866	Yes	0.601	No
9.	Pelargonidin	2.433	No	0.787	No

From the above table 8B, it can be clearly seen that Epicatecin, Chrysin, and Eriodictyol are mutagenic and hence they are toxic to the human body and cannot be used for the further analysis. Many synthetic drugs fail this toxicity determination test, hence it is necessary for every compound to pass this test in order to be used as a drug.

The LD50 values of all the screened flavonoids have been compared with the LD50 values of the FDA approved drugs with the help of a graph (figure 8), depicting that the flavonoids have better LD50 values as compared to the LD50 values of the FDA approved drugs.

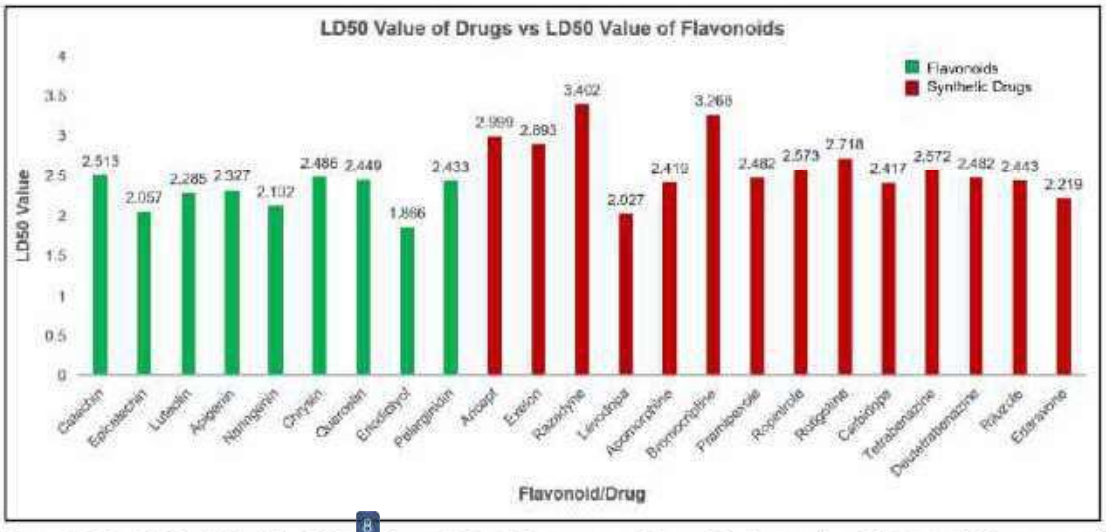

Figure 8: Graph depicting the LD50 values of the FDA approved drugs for the various NDDs and the screened flavonoids.

From the above graph, it can be clearly stated that the screened flavonoids show better LD50 values in comparison to any of the drugs approved for the respective diseases.

4.6 Analysis of the 3-D Structures of the Proteins

The protein that were chosen are:

- Acetylcholinesterase (AChE) for AD
- Dopamine (DA) for PD
- Soluble Carrier Family 22 Member 6 (SCN) for ALS
- Synaptic Vesicular Membrane Transport (SCN) for HD

The proteins were chosen on the basis of the targets of the drugs that were approved by the FDA for the respective diseases. All these targets are the potential targets of their respective diseases. The structures of the proteins were obtained from RCSB PDB (https://www.rcsb.org/) [77]. They were obtained in the ".pdb" format. The structures along with their PDB IDs are depicted in the figure 9.

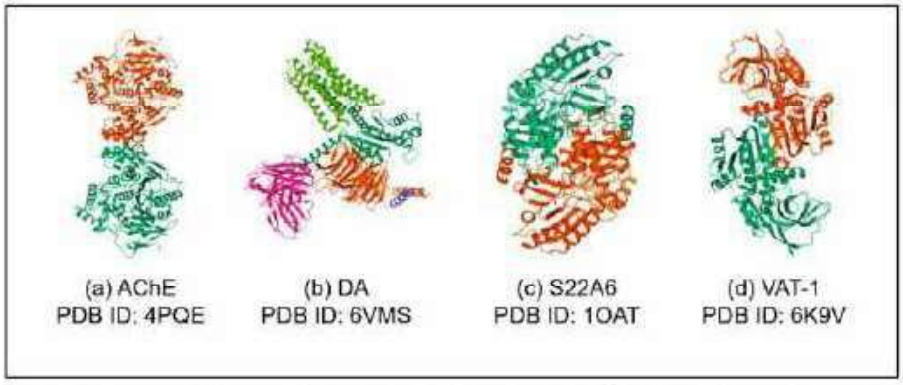

Figure 9: 3-DStructures of the protein targets obtained from the RCSB PDB chosen for the study. 3-D structure of (a) AChE for AD, DA for PD, (c) S22A6 for ALS, and (d) VAT-1 for HD.

4.7 Analysis of the 3-D Structures of Ligands

The ligands were of two types: the conventional drugs that were approved by the FDA and the natural compounds.

The conventional drugs that were approved by the FDA:

- Aricept, Exelon and Razadyne for AD (Figure 10A).
- Levodopa, Apomorphine, Bromocriptine, Pramipexole, Ropinirole, Rotigotine, and Carbidopa for PD (Figure 10B).
- Riluzole and Edaravone for ALS (figure 10C).
- Tetrabenazine and Deutetrabenazine for HD (figure 10D).

The structures of the conventional drugs were obtained from the DrugBank (https://www.drugbank.ca/) [76].

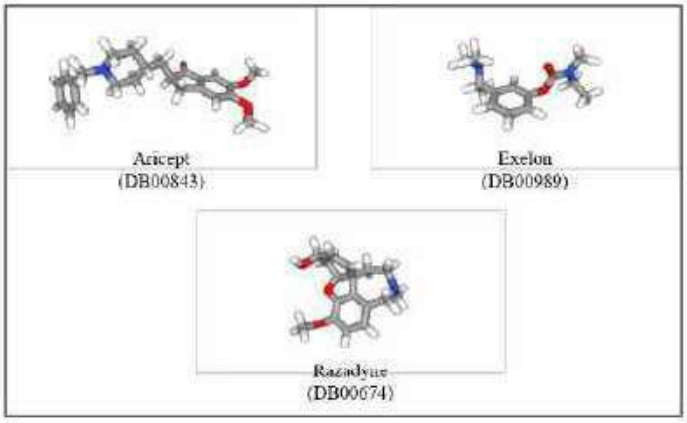

Figure 10A: 3-D Structures of FDA approved drugs for AD.

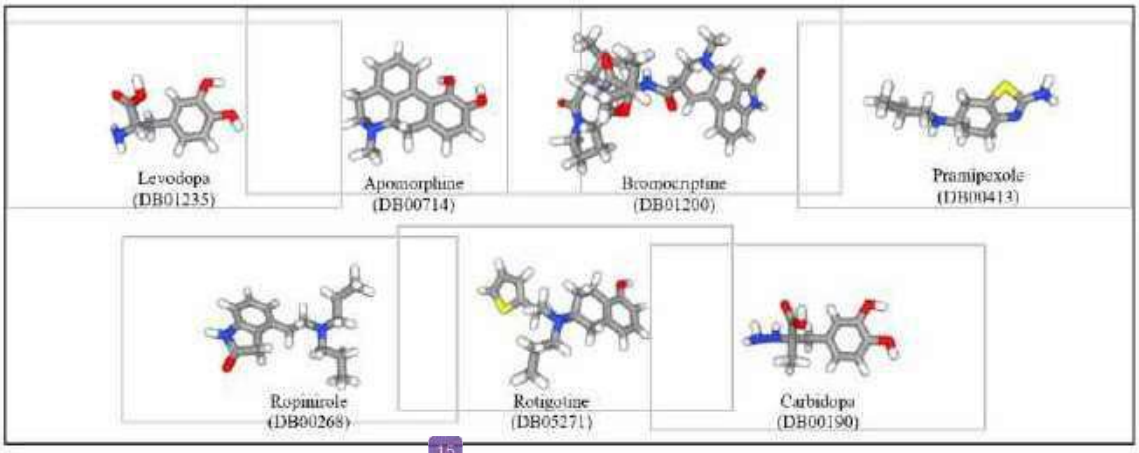

Figure 10B: 3-D Structure of FDA approved drugs for PD.

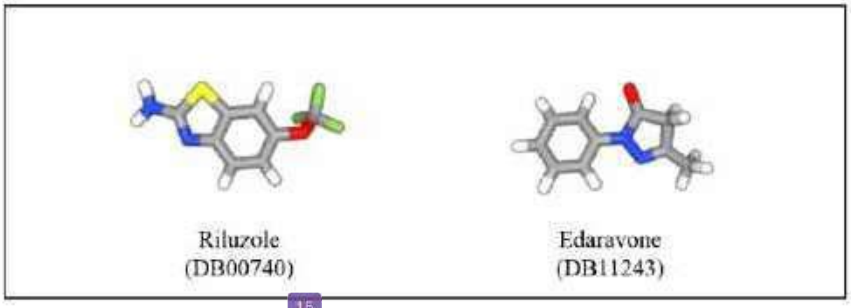

Figure 10C: 3-D Structure of FDA approved drugs for ALS.

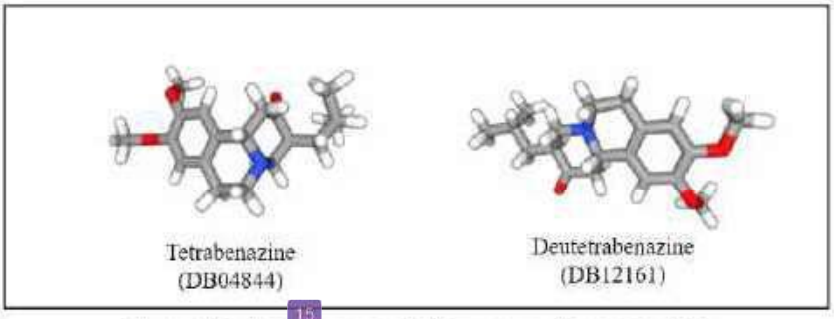

Figure 10D: 3-D Structure of FDA approved drugs for HD.

A total of 06 screened flavonoids (figure 11) were chosen for the molecular docking analysis. Their structures were obtained from the ChEBI (Chemical Entities of Biological Interest, https://www.ebi.ac.uk/chebi/) [75]. The flavonoids that were not available on the ChEBI database, their structures were obtained from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) [74].

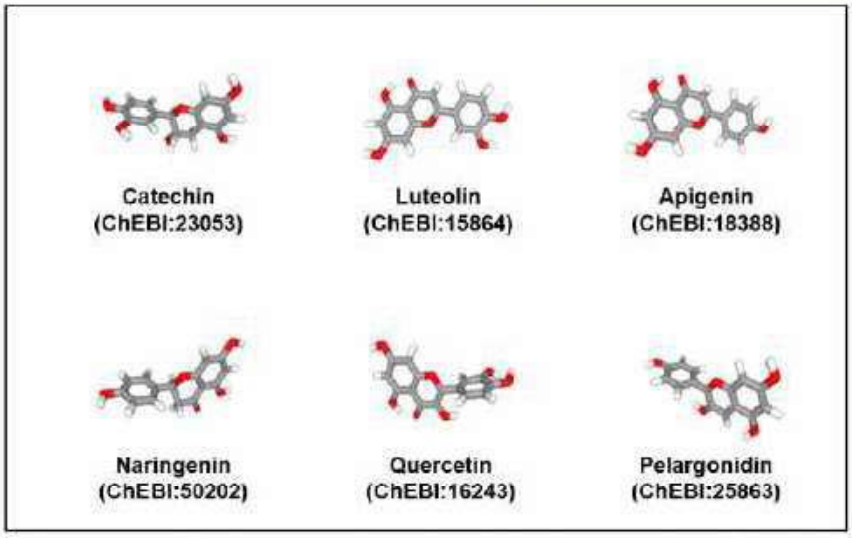

Figure 11: 3-D structure of the six screened flavonoids obtained from the ChEBI (Chemical Entities of Biological Interest) and in some from the PubChem.

4.8 Molecular Docking Studies

Molecular docking is a molecular modelling approach which involves the interaction of two or more molecules and provides stability to the resultant structure [91]. The docking analysis was carried out using the AutoDock Vina in PyRx. The ligands were docked against protein receptors, which were the most potent targets of their respective diseases. The conventional drugs were all

protein inhibitors that has been approved by the FDA for the treatment of the respective diseases and after the analysis obtained from the pharmacokinetics, it can be said that the flavonoids also behaved in a similar way as that of the conventional drugs. Hence, they were also docked against protein targets.

4.8.1 Alzheimer's Disease (AD)

The receptor chosen was AChE. It was docked against the conventional drugs approved by FDA that are Aricpet, Exelon, and Razadyne; and the thirteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The following binding affinities were obtained (table 9):

Table 9: Binding Affinity (kCal/mol) of conventional drugs of AD and flavonoids when docked against AChE.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Aricept (Drug)	-5.0
2.	Exelon (Drug)	-5.91
3.	Razadyne (Drug)	-5.0
4.	Catechin	-8.3
5.	Luteolin	-8.9
6.	Apigenin	-9.1
7.	Naringenin	-8.6
8.	Quercetin	-8.9
9.	Pelargonidin	-7.5

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against AChE, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of AChE. The binding of the ligands with AChE have been illustrated in the Figure 12.

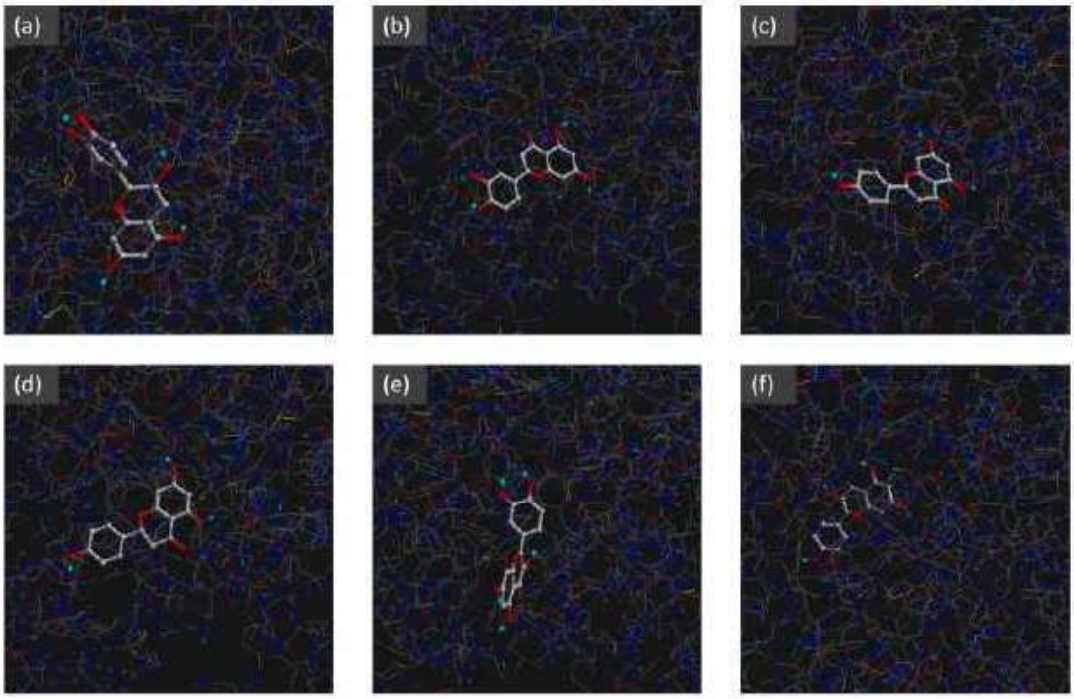

Figure 12: Docking study of Acetylcholinesterase (AChE) with the screened flavonoids. (a) AChE interaction with Catechin, (b)AChE interaction with Luteolin, (c) AChE interaction with Apigenin, (d) AChE interaction with Naringenin, (e) AChE interaction with Quercetin, and (f) AChE interaction with Pelargonidin.

4.8.2 Parkinson's Disease (PD)

The receptor chosen was dopamine. It was docked against the conventional drugs approved by FDA that are Levodopa, Apomorphine, Bromocriptine, Pramipexole, Ropinirole, Rotigotine, and Carbidopa; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 10:

Table 10: Binding Affinity (kCal/mol) of conventional drugs of PD and flavonoids when docked against Dopamine.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Levodopa (Drug)	-6.3
2.	Apomorphine (Drug)	-7.6
3.	Bromocriptine (Drug)	-10.7
4.	Pramipexole (Drug)	-6.1
5.	Ropinirole (Drug)	-6.2

6.	Rotigotine (Drug)	-7.1
7.	Carbidopa (Drug)	-6.2
8.	Catechin	-7.3
9.	Luteolin	-8.3
10.	Apigenin	-8.1
11.	Naringenin	-8.1
12.	Quercetin	-8.6
13.	Pelargonidin	-7.0

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against Dopamine, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of Dopamine. The binding of the ligands with Dopamine have been illustrated in the Figure 13.

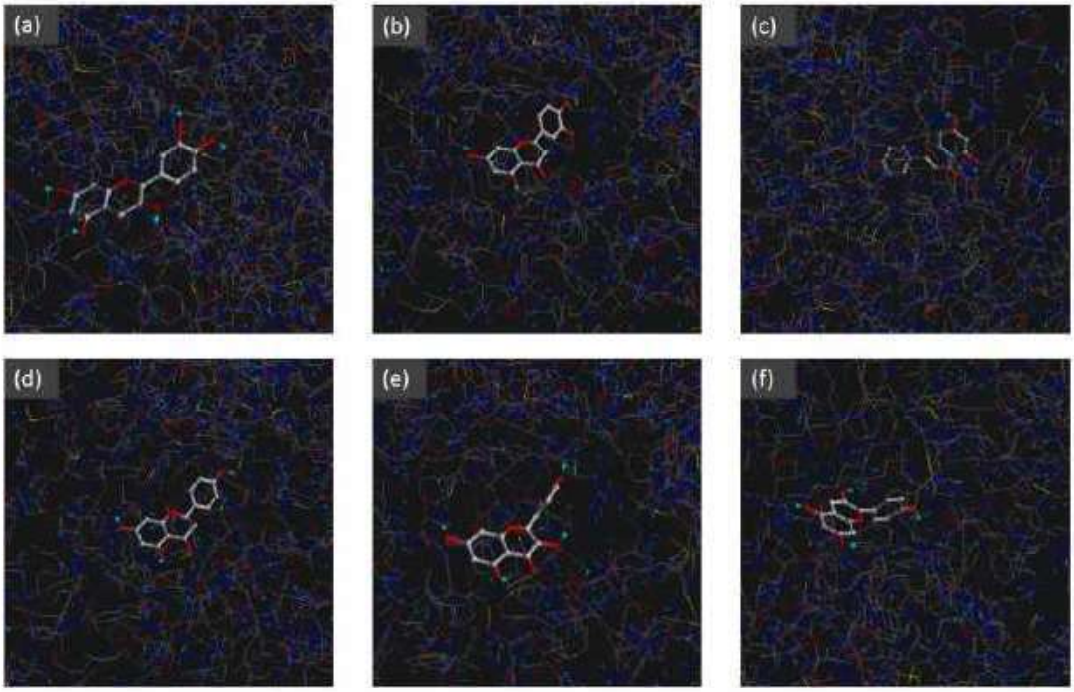

Figure 13: Docking study of Dopamine (DA) with the screened flavonoids. (a) DA interaction with Catechin, (b) DA interaction with Luteolin, (c) DA interaction with Apigenin, (d) DA interaction with Naringenin, (e) DA interaction with Quercetin, and (f) DA interaction with Pelargonidin.

4.8.3 Amyotrophic Lateral Sclerosis (ALS)

The receptor chosen was Soluble Carrier Family 22 Member 6 (S22A6). It was docked against the conventional drugs approved by FDA that are Riluzole and Edaravone; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 11:

Table 11: Binding Affinity (kCal/mol) of conventional drugs of ALS and flavonoids when docked against Soluble Carrier Family 22 Member 6.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Riluzole (Drug)	-6.6
2.	Edaravone (Drug)	-6.7
3.	Catechin	-8.3
4.	Luteolin	-9.2
5.	Apigenin	-8.6
6.	Naringenin	-8.2
7.	Quercetin	-8.5
8.	Pelargonidin	-8.2

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against S22A6, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of S22A6. The binding of the ligands with S22A6 have been illustrated in the Figure 14.

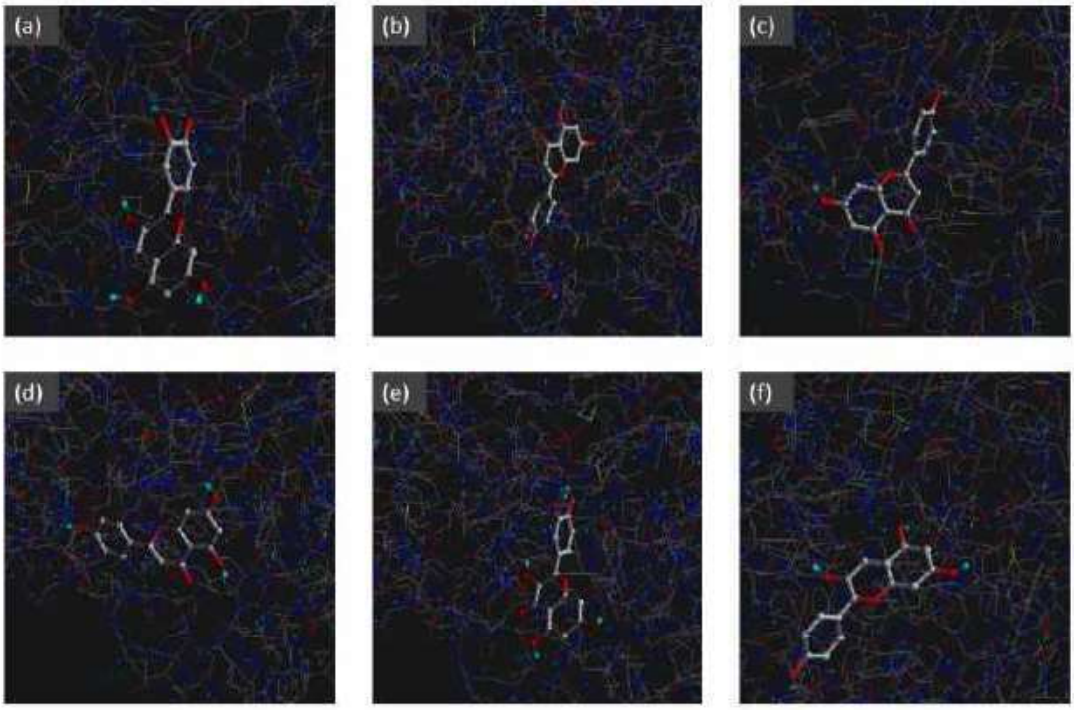

Figure 14: Docking study of Soluble Carrier Family 22 Member 6 (S22A6) with the screened flavonoids. (a) S22A6 interaction with Catechin, (b) S22A6 interaction with Luteolin, (c) S22A6 interaction with Apigenin, (d) S22A6 interaction with Naringenin, (e) S22A6 interaction with Quercetin, and (f) S22A6 interaction with Pelargonidin.

4.8.4 Huntington's Disease

The receptor chosen was Synaptic Vesicular Membrane Transporter (VAT-1). It was docked against the conventional drugs approved by FDA that are Tetrabenazine and Deutetrabenazine; and the fourteen flavonoids that satisfied the ADMET properties. The docking was done using the AutoDock Vina in PyRx. The binding affinities that were obtained are depicted in table 12:

Table 12: Binding Affinity (kCal/mol) of conventional drugs of HD and flavonoids when docked against Synaptic Vesicular Membrane Transporter.

S.No.	Ligand	Binding Affinity (kcal/mol)
1.	Tetradibenazine (Drug)	-6.5
2.	Deutetradibenazine (Drug)	-7.5
3.	Catechin	-8.0
4.	Luteolin	-8.7
5.	Apigenin	-8.4

6.	Naringenin	-9.4
7.	Quercetin	-8.2
8.	Pelargonidin	-7.2

From the above table, it is clearly visible that the flavonoids have binding affinities more than the conventional FDA approved drugs when docked against VAT-1, which is the target of these conventional drugs. The binding affinities of the flavonoids show that they have the potential ability to act as the inhibitor of VAT-1. The binding of the ligands with VAT-1 have been illustrated in the Figure 15.

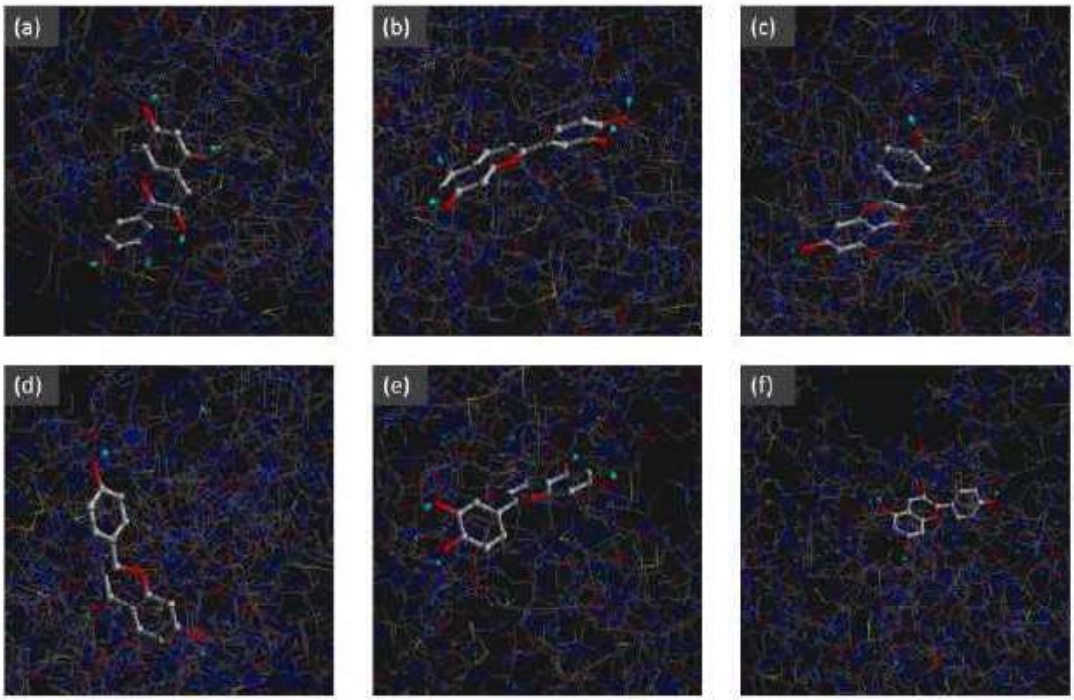

Figure 15: Docking study of Synaptic Vesicular Membrane Transporter (VAT-1) with the screened flavonoids. (a) VAT-1 interaction with Catechin, (b) VAT-1 interaction with Luteolin, (c) VAT-1 interaction with Apigenin, (d) VAT-1 interaction with Naringenin, (e) VAT-1 interaction with Quercetin, and (f) VAT-1 interaction with Pelargonidin.

From the above docking studies of the four diseases, it is clear that there are six flavonoids namely, Catechin, Luteolin, Apigenin, Naringenin, Quercetin and Pealrginidin have shown better binding affinity to the respective targets of the diseases, in comparison to the FDA approved drugs. All these flavonoids belong to different classes of the flavonoids. Catechin and Quercetin belong to

flavonol class; Luteolin and Apigenin belong to the flavone class; Naringenin is a flavonone; and Pelargonidin is a anthocyanidin. All these can be obtained from a number of herbs and spices. Hence, these flavonoids can be used as whole or in combination with other flavonoids to make a drug that can be used for the treatment of the NDDs.

4.9 Prediction of Bioactivity Score

The bioactivity score of the compounds were predicted using the Molinspiration Software.

Table 13: Bioactivity Score of the Flavonoids predicted using the Molinspiration Software.

S.No.	Flavonoid	GPCR Ligand (GPCR)	Ion Channel Modulator (ICM)	Kinase inhibitor (KI)	Nuclear Receptor Ligand (NRL)	Protease Inhibitor (PI)	Enzyme Inhibitor (EI)
1.	Catechin	0.41	0.14	0.09	0.60	0.26	0.47
2.	Luteolin	-0.02	-0.07	0.26	0.39	-0.22	0.28
3.	Apigenin	-0.07	-0.09	0.18	0.34	-0.25	0.26
4.	Naringenin	0.03	-0.20	-0.26	0.42	-0.12	0.21
5.	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
6.	Pelargonidin	-0.18	-0.11	-0.07	0.03	-0.33	-0.02

From table 13, it can be clearly seen that catechin has bioactivity score >0.0 for all the parameters, hence it is highly active. Luteolin, apigenin, and quercetin have bioactivity score between 0.0 and -0.5, for the parameters, GPCR, ICM, and PI, hence show moderate activities against these parameters. But, they show high activities against the parameters KI, NRL, and EI as their bioactivity score is more than 0.0. Naringenin show moderate activities against ICM, KI, and PI (bioactivity score between 0.0 and -0.5) and high activity against the GPCR, NRL, and EI (bioactivity score >0.0). Pelargonidin is moderately active for all the parameters (bioactivity score between 0.0 and -0.5), except for NRL. Nuclear receptor ligands (NRLs) are important pharmaceutical targets due to their function as key regulators of many metabolic and inflammatory diseases, including diabetes, dyslipidemia, cirrhosis and fibrosis [89]. From the above table, it can be clearly seen that the flavonoids show high activity against NRL (bioactivity score >0.0).

4.10 In silico determination of antioxidant activity of the screened flavonoids

The antioxidant activity of the screened flavonoids were determined using the *in silico* analysis. The software used for the antioxidant analysis was the PASS online server. The parameters for the antioxidant activity of the screened flavonoids have been depicted in the table 14.

Table 14: Antioxidant Activity of the screened flavonoids using PASS online.

S.No.	Flavonoids	Pa Antioxidant	Pa Free Radical Scavenger	Pa Anticarcinogenic
1.	Catechin	0.810	0.842	0.795
2.	Luteolin	0.775	0.755	<0.7
3.	Apigenin	0.732	0.719	<0.7
4.	Narigenin	0.794	0.769	0.724
5.	Quercetin	0.872	0.811	0.757
6.	Pelargonidin	NA	NA	NA

To use any compound as a drug, the compound has to fulfill certain conditions. From the above table, it can be seen that all the screen flavonoids have Pa values to act as an antioxidant more than 0.7 and hence they can be used as drugs (as whole or in combination with other flavonoids). The prediction could not be carried out for the Pelargonidin because it has a molecular charge of +1.

5. CONCLUSION

Flavonoids are secondary metabolites present in plants and hence have no or lesser. They are also present in the herbs and spices that we use in our day-to-day lives in our Indian households and hence are easily available for use, in comparison to the conventional drugs. We started with twenty-seven flavonoids and after predicting their molecular properties, drug-likeness properties, and pharmacokinetic properties we were left with six flavonoids. Their toxicity was also determined, in which the flavonoids proved to be less toxic in comparison to the FDA approved drugs as they were neither carcinogenic nor harmful to the liver in the long run. The LD50 values of the flavonoids were also less than the FDA approved drugs, proving them to be less toxic. These six flavonoids when docked with targets of the respective diseases, we came to a conclusion that all the screened flavonoids, Catechin, Luteolin, Apigenin, Naringenin, Quercetin, and Pelargonidin showed better binding affinities with the drug targets in comparison to the conventional drugs itself. To use these flavonoids in the form of drugs they can either be used as a whole or in combination with other flavonoids. To know the efficacy of the flavonoids as a drug, their bioactive score was predicted, in which all the flavonoids were highly or moderately active. With the help of this *in* silico study, we could just find out how, herbs and spices that are present in the Indian households can be proved effective to be used for the treatment of the NDDs.

Neurodegenerative diseases

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		Credits	3	3	3	3	3	2	2	1	20		Papers Failed
1	ASMITA JAISWAL	2K18/BME/01	O	B+	B+	B+	B+	A	A+	O	20	7.90	
2	SHALEEN JAIN	2K18/BME/02	A+	B+	A	B+	B+	A+	A+	A+	20	7.95	
3	SHRUTI THAREJA	2K18/BME/03	A+	B+	A	B+	B+	A	A+	O	20	7.90	

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BT-663 : MEDICAL AND TISSUE ENGINEERING LAB
BT-664 : MINOR PROJECT-I

Sr.No	Name	Roll No.	BT-611	BT-6121	BT-6131	BT-614	BT-6611	BT-6622	BT-663	BT-664	TC	SGPA	Papers Failed
		Credits	3	3	3	2	3	3	2	1			
1	ASMITA JAISWAL	2K18/BME/01	C	B	A+	B	B+	A	A+	B+	20	7.10	
2	SHALEEN JAIN	2K18/BME/02	A	A	A	B+	A	A	A+	A	20	8.00	
3	SHRUTI THAREJA	2K18/BME/03	B	A	A	B+	A	B+	A+	A	20	7.55	

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Sem : III

Branch : Bio Medical Engineering

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BT-7111 : BIOETHICS AND IPR BT-7122 : BIOINFORMATICS BT-713 : SELF STUDY OPEN AREA SEMINAR-II BT-714 : MINOR PROJECT II

BT-715 : MAJOR PROJECT I

Sr.No	Name	Roll No.	BT-7111	BT-7122	BT-713	BT-714	BT-715	TC	SGPA	Papers Failed
		Credits	3	3	2	6	6			
1	ASMITA JAISWAL	2K18/BME/01	A	A	A+	A+	A	20	8.40	
2	SHALEEN JAIN	2K18/BME/02	A+	B+	A+	O	A+	20	9.00	
3	SHRUTI THAREJA	2K18/BME/03	A+	A	A+	O	A	20	8.85	

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