IN-SILICO APPROACHES OF POLYPHENOLS AND *IN -VIVO* EVALUATION OF NEUROPROTECTIVE EFFECTS *OF EUGENIA JAMBOLANA* LEAVES EXTRACT FOR ANTICHOLINESTERASE AND ANTIOXIDANT ACTIVITIES

A Dissertation submitted to

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY
CHENNAI- 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH-VI- PHARMACOLOGY

Submitted by

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October 2021

CERTIFICATES

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CERTIFICATE

This is to certify that the dissertation entitled "***IN-SILICO* APPROACHES OF POLYPHENOLS AND *IN- VIVO* EVALUATION OF NEUROPROTECTIVE EFFECTS OF *EUGENIA JAMBOLANA* LEAVES EXTRACT FOR ANTICHOLINESTERASE AND ANTIOXIDANT ACTIVITIES"** is a Bonafied research work done by **Ms SREYA S (Reg. No: 261926153)** in partial fulfillment for the award of the degree of **Master of Pharmacy in Pharmacology.** The Research work was carried out in **Department of Pharmacology, Karpagam College of Pharmacy** and submitted to **The Tamilnadu Dr. M.G.R. Medical University,Chennai** under the supervision and guidance of **Mr.N.SUNDARARAJAN M. Pharm** during the academic year 2020-2021.The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

PRINCIPAL

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RESEARCH GUIDE

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I hereby declare that this dissertation work entitled "***IN-SILICO* APPROACHES OF POLYPHENOLS AND *IN- VIVO* EVALUATION OF NEUROPROTECTIVE EFFECTS OF *EUGENIA JAMBOLANA* LEAVES EXTRACT FOR ANTICHOLINESTERASE AND ANTIOXIDANT ACTIVITIES"** submitted by me ,in partial fulfillment for the award of the degree of **Master of Pharmacy in Pharmacology** to **The Tamilnadu Dr. M.G.R. Medical University, Chennai** is the result of my original and independent research work carried out under the guidance of **Mr.N.SUNDARARAJAN M.Pharm ,Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore** during the academic year 2020-2021.

The work is original and the dissertation either in part or full has not been submitted by me or any other persontoanyUniversity/Instituteinanypartofthesis/dissertation/monograph.

I hereby further declare that the **Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic or research purpose.

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EVALUATION CERTIFICATE

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ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

Apart from my effort, the success if this dissertation mainly depends on theencouragement and guidance of many others. I take this opportunity to express my gratitudeto the people who have been instrumental behind the successful completion of this dissertation work.

First and foremost, I wish to express my thanks to Holiness for his love and unseen guidance throughout my work.

I would like to express my profound thanks to my beloved Parents, Father **Mr Suresh Kumar** and Mother **Mrs. Latha K K** who brought me to this world and encouragements throughout my life journey and my Husband **Mr Rathnaj P S** for his support and encouragements in all these two years, and to my Brother **Sreedharsh** and his Wife **Rehna Sreedharsh** for their support and help they offered in these two years

Firstly, I extended thanks to **Dr.Vasanthakumar, Chairman, Karpagam Academy of Higher Education, Coimbatore** for the facilities provided by him to carry out the study in efficient manner.

I proudly take this opportunity to express my deep sense of gratitude to **Dr.S.Mohan, M.Pharm, Ph.D., Principal, Karpagam College of pharmacy, Coimbatore,** for his invaluable encouragement to complete thisproject.

I am particularly thankful to **Mr.N.SUNDARARAJAN M. Pharm ,Associate Professor, Department of Pharmacology, Karpagam College of Pharmacy,**for his heartily and valuable guidance during the course of my work.

I express my sincere thanks to **Dr.C Senthil kumar , M Phram ,PGDCR, Phd ,Professor & Head** , **Department of Pharmacology, Karpagam College of Pharmacy,** for his valuable support through out the project.

I express my sincere thanks to **Mrs. Swarnakumari** , **M Pharm**, **Associate Professor Ms Mumtha L, Assistant Professor Department of Pharmacognosy, Karpagam College of Pharmacy,** for their guidance in plant extraction and Estimation of Total phenolic content

I express my sincere thanks to **Dr. M Sasikala M Pharm, Associate Professor, Department of Pharmaceutical Chemistry, Karpagam College of pharmacy,**for his valuable guidance in HPTLC procedures .

I express my sincere thanks to **Ms S.Grace M.Pharm** and **Ms.T.Vaishnavi.M.Sc**, for their valuable guidance in ligand structure and statistical analysis.

I also convey our gratitude to **Librarian and Lab Technician of Karpagam College of Pharmacy** and all those helped me in the completion of my entire work.

I want to express my gratitude to **My friends and Juniours of Karpagam College of Pharmacy** for helped me in the completion of my entire work.

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LIST OF ABBREVATION

+	Positive
-	Negative
<	Less Than
>	Greater Than
=>	Greater Than Equal
<=	Less Than Equal
Mg	MilliGram
µg	Micro Gram
Kg	Kilo gram
AD	Alzheimer's Disease
MCI	Mild Cognitive Impairment
U.S	United States
SP	Senile Plaques
Aβ	Amyloid Beta
APP	Precursor Protein Of Amyloid
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
DNA	DeoxyRibonucleolic Acid
RNA	Ribo Nucleic Acid
GAE	Galic acid equivalent
DTNB	DIthio-bis-2-nitrobenzoic acid
APOE	Apo-Lipo-Protein E
Ach	Acetyl Choline
Ache	Acetyl-Cholinesterase
Bche	Butrylcholinesterase
Cheis	Cholinesterase Inhibitors
Acheis	Acetyl-Cholinesterase Inhibitors
TBI	Traumatic Brain Injury
CT	Computerized Tomography
PET	Positron Emission Tomography
FDG	Fluorodeoxyglucose
CYP2D6	Cytochrome P2D6
CYP 3A4	Cytochrome P3A4
BID	Bis In Die
FAD	Familial Alzheimer's Disease
NMDA	N-Methyl-D-Aspartate Receptor
Bm	BacopaMonnieri
CC	Cat’s Claw
Gb	*Ginkgo Biloba*
Gk	GotuKola
Cp	Convolvulus Pluricaulis
DMC	Demethoxycurcumin
BDMC	Bisdemethoxycurcumin
Ca	Calcium
Mg	Magnesium

Fe	Ferric
Na	Sodium
K	Potassium
CADD	Computer Aided Design And Drafting
ADMET	Absorption distribution metabolism excretion toxicity
IAEC	Institutional animal ethical committee
HPTLC	High Performance Thin Layer Chromatography
DPPH	diphenyl-1-picrylhydrazyl
MW	Molecular weight
RO5	Rule of five
CNS	Central nervous system
VDs	Volume of distribution
Pgp	P-glycoprotein
BBB	Blood brain Barrier
LOAEL	Lowest Observed Adverse effect
LD 50	Lethal Dose
ICG 50	Inhibition Growth Concentration
OECD	Organization For Economic Cooperation and Development
SD rats	Sprague–Dawley rats
RIVA	Rivastigmine
ANOVA	Analysis of variance
OCT2	Organic Cation Transporter 2
ABC	ATP-binding Cassette
MRTD	Maximum Recommended tolerated Dose

1.INTRODUCTION

1.1 Alzheimer's

Alzheimer's disease is a progressive neurologic disorder that causes the brain to shrink (atrophy) and brain cells to die. Alzheimer's disease is the most common cause of dementia — a continuous decline in thinking, behavioral and social skills that affects a person's ability to function independently.[2]

Dimentia is an overall term for a particular group of symptoms. The characteristic symptoms of dementia are difficulties with memory, language, problem-solving and other thinking skills that affect a person's ability to perform everyday activities. Dementia has many causes . Alzheimer's disease is the most common cause of dementia.[1]

The disease is named after Dr. Alois Alzheimer. In 1906, Dr. Alzheimer noticed changes in the brain tissue of a woman who had died of an unusual mental illness After she died, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called neurofibrillary, or tau, tangles). These plaques and tangles in the brain are still considered some of the main features of Alzheimer's disease. [2,4]Another feature is the loss of connections between nerve cells (neurons) in the brain. Neurons transmit messages between different parts of the brain, and from the brain to muscles and organs in the body. Many other complex brain changes are thought to play a role in Alzheimer's, too.

This damage initially takes place in parts of the brain involved in memory, including the entorhinal cortex and hippocampus. It later affects areas in the cerebral cortex, such as those responsible for language, reasoning, and social behavior. Eventually, many other areas of the brain are damaged.[3]

1.2 Brain changes associated with Alzheimer's disease

A healthy adult brain has about 100 billion neurons, each with long, branching extensions. These extensions enable individual neurons to form connections with other neurons. At such connections, called synapses, information flows in tiny bursts of chemicals that are released by one neuron and detected by another neuron.[3] The brain contains about 100 trillion synapses.

They allow signals to travel rapidly through the brain's neuronal circuits, creating the cellular basis of memories, thoughts, sensations, emotions, movements and skills.

The accumulation of the protein fragment beta-amyloid (called beta-amyloid plaques) *outside* neurons and the accumulation of an abnormal form of the protein tau (called tau tangles) *inside* neurons are two of several brain changes associated with Alzheimer's.[4]

Plaques and smaller accumulations of beta-amyloid called oligomers may contribute to the damage and death of neurons (neurodegeneration) by interfering with neuron-to-neuron communication at synapses. Tau tangles block the transport of nutrients and other essential molecules inside neurons. Although the complete sequence of events is unclear, beta-amyloid may begin accumulating before abnormal tau, and increasing beta-amyloid accumulation is associated with subsequent increases in tau.[5]

Other brain changes include inflammation and atrophy. The presence of toxic beta-amyloid and tau proteins are believed to activate immune system cells in the brain called microglia. Microglia try to clear the toxic proteins as well as widespread debris from dead and dying cells. Chronic inflammation may set in when the microglia can't keep up with all that needs to be cleared. Atrophy, or shrinkage, of the brain occurs because of cell loss. Normal brain function is further compromised in Alzheimer's disease by decreases in the brain's ability to metabolize glucose, its main fuel.[6]

1.3 Types of Alzheimer's Disease

1.3.1 Classification Based on the Severity

Based on the intensity of the typical Alzheimer's symptoms, it can be classified into the,

Mild Alzheimer's

This includes the beginning of cognitive impairment that causes difficulties in remembering daily routine such as tasks at work, paying bills , and others. Because these symptoms are not

very serious, the patients at this stage manage to remain functional with a certain amount of difficulty. They take longer to perform the same task which theyused to do quicker before, and this becomes a pattern.

Moderate Alzheimer's

Because of a significant amount of neuronal damage, the symptoms of moderate Alzheimer's are more intense. The confusion becomes worse and due to the amount of memory loss, they become increasingly dependent on others. These individuals, even though physically agile, are not able to perform routine tasks as the delusions take over the sensory processing of their thoughts.[7]

Severe Alzheimer's

As the plaques and tangles spread, the brain cells start dying. This results in shrinkage of brain tissue. The patients with this condition are typically bedridden and are hardly able to communicate.[7]

These subtypes are more like stages of the disease, and it often progresses from a milderto a more severe form. The sooner the patient is diagnosed with the condition, the better are the chances of treating and preventing its progression. [8]

1.3.2 Classification Based on the Inflammatory Response

Alzheimer’s is categorized into three subtypes based on inflammatory response:

Inflammatory :In addition to the behavioral and cognitive symptoms, this subtype exhibits high serum albumin to globulin ratio and high level of C-reactive protein in response to inflammation.

Non-Inflammatory :This subtype of Alzheimer's does not exhibit elevated inflammatory biomarkers. However, other metabolic abnormalities are usually associated with this condition.

Cortical :A cortical subtype is caused by a deficiency of zinc throughout various regions of the brain. Even though there is no inflammatory response associated with this subtype, it causes abnormalities in normal brain functioning which lead to Alzheimer's disease.[7]

1.3.3 Classification Based on the Onset or Trigger Type

Early-Onset Alzheimer's

The subtype of Alzheimer's disease affecting people below 65 years of age is referred to as early-onset Alzheimer's. This condition is very rare (5 out of 100 Alzheimer's patients). The changes usually happen when the patients reach their late 40s or early 50s. Distinct features of this condition are considered the outcome of a defect in Chromosome 14.[19]

Late-Onset Alzheimer's

Majority of Alzheimer's cases are late-onset, affecting people older than 65 years of age. The exact genetic trigger is not yet identified. Yet, several risk factors have been named by scientists, and further research is ongoing.[8,9]

Another subcategory of the condition is familial Alzheimer's disease (FAD). This condition is very rare (1 out of 100 cases). A person can only be diagnosed with FAD when the specific genotypic pattern of illness is characterized by the family members, and exact risk can be predicted.

Alzheimer's disease can take a toll on the patients cognitive, physical, and social abilities. The early detection of the disease is crucial in preventing or slowing down its progression.[9]

1.4 Alzheimer's stages: How the disease progresses

Alzheimer's disease tends to develop slowly and gradually worsens over several years. Eventually, Alzheimer's disease affects most areas of your brain. Memory, thinking, judgment, language, problem-solving, personality and movement can all be affected by the disease.

There are five stages associated with Alzheimer's disease: preclinical Alzheimer's disease, mild cognitive impairment due to Alzheimer's disease, mild dementia due to Alzheimer's disease, moderate dementia due to Alzheimer's disease and severe dementia due to Alzheimer's disease. Dementia is a term used to describe a group of symptoms that affect intellectual and social abilities severely enough to interfere with daily function.[10]

1.4.1 Preclinical Alzheimer's disease

Alzheimer's disease begins long before any symptoms become apparent. This stage is called preclinical Alzheimer's disease, and it's usually identified only in research settings. You won't notice symptoms during this stage, nor will those around you.

This stage of Alzheimer's can last for years, possibly even decades. Although you won't notice any changes, new imaging technologies can now identify deposits of a protein called amyloid-beta that is a hallmark of Alzheimer's disease. The ability to identify these early deposits may be especially important for clinical trials and in the future as new treatments are developed for Alzheimer's disease.[11]

Additional biomarkers — measures that can indicate an increased risk of disease — have been identified for Alzheimer's disease. These biomarkers can be used to support the diagnosis of Alzheimer's disease, typically after symptoms appear.

Genetic tests also can tell you if you have a higher risk of Alzheimer's disease, particularly early-onset Alzheimer's disease. These tests aren't recommended for everyone, but you and your doctor can discuss whether genetic testing might be beneficial for you.

As with newer imaging techniques, biomarkers and genetic tests will become more important as new treatments for Alzheimer's disease are developed.[12]

1.4.2 Mild cognitive impairment (MCI) due to Alzheimer's disease

People with mild cognitive impairment have mild changes in their memory and thinking ability. These changes aren't significant enough to affect work or relationships yet. People with MCI may have memory lapses when it comes to information that is usually easily remembered, such as conversations, recent events or appointments.

People with MCI may also have trouble judging the amount of time needed for a task, or they may have difficulty correctly judging the number or sequence of steps needed to complete a task. The ability to make sound decisions can become harder for people with MCI.

Not everyone with mild cognitive impairment has Alzheimer's disease. MCI is often diagnosed based on the doctor's review of symptoms and professional judgment. But if necessary, the same procedures used to identify preclinical Alzheimer's disease can help determine whether MCI is due to Alzheimer's disease or something else.[13]

1.4.3 Mild dementia due to Alzheimer's disease

Alzheimer's disease is often diagnosed in the mild dementia stage, when it becomes clear to family and doctors that a person is having significant trouble with memory and thinking that impacts daily functioning.[14]

In the mild dementia stage, people may experience:

- **Memory loss of recent events.** Individuals may have an especially hard time remembering newly learned information and ask the same question over and over.
- **Difficulty with problem-solving, complex tasks and sound judgments.** Planning a family event or balancing a checkbook may become overwhelming. Many people experience lapses in judgment, such as when making financial decisions.
- **Changes in personality.** People may become subdued or withdrawn — especially in socially challenging situations — or show uncharacteristic irritability or anger. Reduced motivation to complete tasks also is common.

- **Difficulty organizing and expressing thoughts.** Finding the right words to describe objects or clearly express ideas becomes increasingly challenging.
- **Getting lost or misplacing belongings.** Individuals have increasing trouble finding their way around, even in familiar places. It's also common to lose or misplace things, including valuable items.[15]

1.4.4 Moderate dementia due to Alzheimer's disease

During the moderate dementia stage of Alzheimer's disease, people grow more confused and forgetful and begin to need more help with daily activities and self-care.

People with the moderate dementia stage of Alzheimer's disease may:

- **Show increasingly poor judgment and deepening confusion.** Individuals lose track of where they are, the day of the week or the season. They may confuse family members or close friends with one another or mistake strangers for family.

  They may wander, possibly in search of surroundings that feel more familiar. These difficulties make it unsafe to leave those in the moderate dementia stage on their own.

- **Experience even greater memory loss.** People may forget details of their personal history, such as their address or phone number, or where they attended school. They repeat favorite stories or make up stories to fill gaps in memory.
- **Need help with some daily activities.** Assistance may be required with choosing proper clothing for the occasion or the weather and with bathing, grooming, using the bathroom and other self-care. Some individuals occasionally lose control of their bladder or bowel movements.
- **Undergo significant changes in personality and behavior.** It's not unusual during the moderate dementia stage for people to develop unfounded suspicions — for example, to become convinced that friends, family or professional caregivers are stealing from them or that a spouse is having an affair. Others may see or hear things that aren't really there.

  Individuals often grow restless or agitated, especially late in the day. Some people may have outbursts of aggressive physical behavior.[16]

1.4.5 Severe dementia due to Alzheimer's disease

In the late stage of the disease, called severe dementia due to Alzheimer's disease, mental function continues to decline, and the disease has a growing impact on movement and physical capabilities.

In late stage severe dementia due to Alzheimer's disease, people generally:

- **Lose the ability to communicate coherently.** An individual can no longer converse or speak in ways that make sense, although he or she may occasionally say words or phrases.
- **Require daily assistance with personal care.** This includes total assistance with eating, dressing, using the bathroom and all other daily self-care tasks.
- **Experience a decline in physical abilities.** A person may become unable to walk without assistance, then unable to sit or hold up his or her head without support. Muscles may become rigid and reflexes abnormal. Eventually, a person loses the ability to swallow and to control bladder and bowel functions.[17]

1.4.6 Rate of progression through Alzheimer's disease stages

The rate of progression for Alzheimer's disease varies widely. On average, people with Alzheimer's disease live between three and 11 years after diagnosis, but some survive 20 years or more [18].The degree of impairment at diagnosis can affect life expectancy. Untreated vascular risk factors such as hypertension are associated with a faster rate of progression of Alzheimer's disease. [19]

1.5 Epidemiology

In 2016, approximately 47 million people live with dementia across the globe. Alzheimer's is the single most common cause of dementia, comprising 70% of all cases. The majority of the patients with Alzheimer's have late-onset (around 65 years of age or later), and few have early-onset during 40’s or 50’s.[7]

Geographical distribution of Alzheimer's is slightly skewed. The western European countries and North America has the highest prevalence of Alzheimer's, followed by China, Latin America, and Western-Pacific countries. The incidence rates also depict similar picture, except for the fact that Latin America has a relatively higher incidence of Alzheimer's compared to the western European countries.

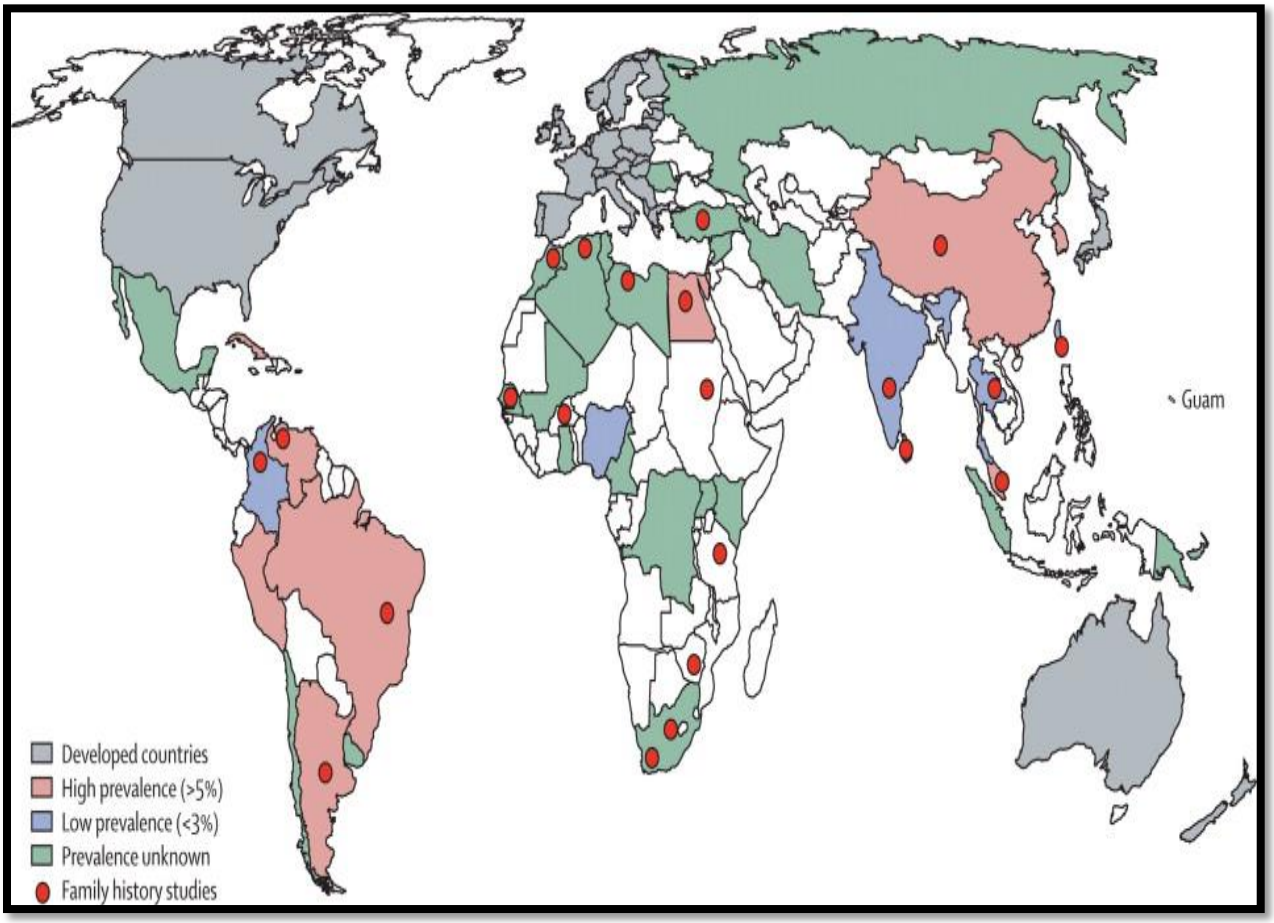


Figure 1: **Sporadic and familial dementias in developing countries [83]**

Out of the total U.S. population:

Prevalence of Alzheimer's and other dementias in the United States

In the United States, approximately 5.7 million people are living with Alzheimer's. It's the 6th leading cause of death in the United States, and the number of deaths skyrocketed by 123% between 2000 and 2015

- One in 10 people (10%) age 65 and older has Alzheimer's dementia.[3]
- The percentage of people with Alzheimer's dementia increases with age: 3% of people age 65-74, 17% of people age 75-84, and 32% of people age 85 and older have Alzheimer's dementia. People younger than 65 can also develop Alzheimer's dementia, but it is much less common and prevalence is uncertain[7,8]

In india between 2001 and 2011, India's elderly population increased from 70 million to 104 million (Census estimates). In 2011, the population over 60 years of age comprised 8.6% of the total population. [7]With falling population growth rates this share is only expected to increase further in the coming decades. As the population ages, the burden of geriatric diseases will start to feel heavier. Of all the geriatric diseases, India is perhaps most underprepared to tackle the burden of degenerative diseases like dementia (memory loss). This is due to a lack of awareness compounded by a dearth of specialists in geriatric diseases.

According to the Dementia India Report 2010 by the Alzheimer's and Related Disorders Society of India (ARDSI), there were around 3.7 million Indians with dementia in 2010 with the number projected to rise to 7.6 million by 2030[9]

1.6 Pathophysiology of Alzheimers

1.6.1 Hyperphosphorylated tau protein and amyloid β hypothesis

One of the main pathological features of AD is the formation of senile plaques (SP), which is caused by amyloid beta (Aβ) deposition. Normally, Aβ are soluble small peptides, which are produced by the splitting of the precursor protein of amyloid (APP) by the action of α-secretase, β-secretase and γ-secretase. The imbalance between β-amyloid (Aβ) production and clearance leads to various types of toxic oligomeric, namely protofibrils, fibrils and plaques depending

upon the extent of oligomerization. The reason of the formation of Aβ is still unclear, but the sequence, concentration and conditions of stability of Aβ are important factors. The pathophysiology of Alzheimer's disease is credited to a number of factors such as the cholinergic dysfunction, amyloid/tau toxicity and oxidative stress/mitochondrial dysfunctions.[20,21]

1.6.2 Oxidative stress hypothesis

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in many normal and abnormal processes in humans, they play dual role as both have beneficial functions in cellular signaling pathways and venomous processes that can lead to damage of cellular structures (including cell membrane, lipid, protein, and DNA). [22]The high oxygen consumption of the brain, which utilizes 20% more oxygen than other mitochondrial respiratory tissues, means that the brain is more vulnerable to oxidative stress. The neuron is the basic functional unit of the brain, which contains a large number of polyunsaturated fatty acids. It can interact with ROS, leading to the lipid peroxidation reaction and molecular apoptosis, in addition, less glutathione in neurons is also one of the causes of oxidative stress injury[23]

1.6.3 Metal ion hypothesis

Metal dyshomeostasis is involved in the progression and pathogenesis of diseases, including neurodegerative diseases and cancer. Ionosphere and metal chelators are well known modulators of transition metal homeostasis, and a number of these molecules are used in clinical trials.

Metal-binding compounds are not the only drugs capable of targeting transition metal homeostasis. Current evidence indicates changes in the equilibrium of redox transition metals; mainly copper (Cu), iron (Fe) and other trace metals. Their levels in the brain are found to be high in AD. In other neurodegenerative disorders, Cu, manganese, aluminum and zinc are involved[24,25]

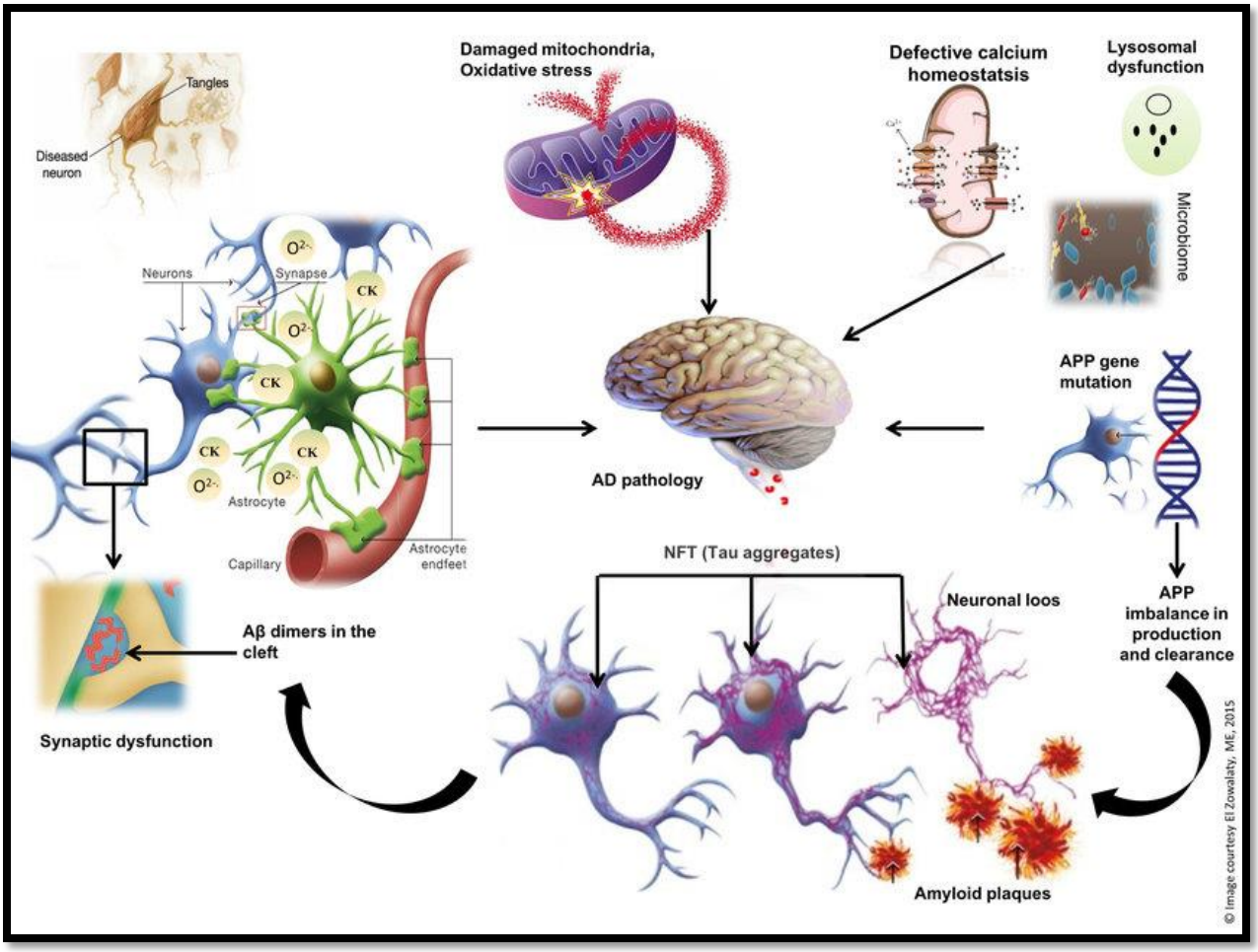


Figure 2: Pathogenesis of Alzheimers disease[28]

1.6.4 Cholinergic hypothesis

The effects of apo-lipo-protein E (APOE) genotype on the useful effect of acetyl-cholinesterase inhibitors (AChEIs) in patients with Alzheimer's disease. AchEI medications are the core of the treatment of AD, and APOE genotype is the most important factor associated with AD. This lack of major effect of APOE is analyzed with respect to the "Cholinergic Hypothesis" of AD, dating from 1976, through the recognition that cholinergic neurons are not the main target of AD.[26]

Cholinergic receptor binding is reduced in specific brain regions with mild to moderate AD and is related to neuropsychiatric symptoms. Among healthy older adults, lower receptor binding may be associated with slower processing speed.[27]

Cholinergic receptor binding in vivo may reveal links to other key brain changes associated with aging and AD and may provide a potential molecular treatment target. Clinical decrease is related to an extensive loss of cholinergic neurons formed in the forebrain nuclei (medial) and a related decline in acetylcholine-mediated neurotransmission, drugs tending to regularize acetylcholine transmitter level, such as cholinesterase inhibitors (ChEIs) and donepezil, have for over 20 years served as the foundation of symptomatic th erapy for AD.[28]

1.7 Signs Of Alzheimer's Dementia

Memory loss that disrupts daily life: One of the most common signs of Alzheimer's dementia is memory loss, especially forgetting recently learned information. Others include forgetting important dates or events, asking for the same information over and over, and increasingly needing to rely on memory aids (for example, reminder notes or electronic devices) or family members for things that used to be handled on one's own

Challenges in planning or solving problems: Some people experience changes in their ability to develop and follow a plan or work with numbers. They may have trouble following a familiar recipe, keeping track of monthly bills or counting change. They may have difficulty concentrating and take much longer to do things than they did before

Difficulty completing familiar tasks at home, at work or at leisure: People with Alzheimer's often find it hard to complete daily tasks. Sometimes, people have trouble driving to a familiar location, managing a budget at work or remembering the rules of a favorite game.[7]

Confusion with time or place: People with Alzheimer's can lose track of dates, seasons and the passage of time. They may have trouble understanding something if it is not happening immediately. Sometimes they forget where they are or how they got there.

Trouble understanding visual images and spatial relationships: For some people, having vision problems is a sign of Alzheimer's. They may have difficulty reading, judging distance and determining color or contrast, which may cause problems with driving.

New problems with words in speaking or writing: People with Alzheimer's may have trouble following or joining a conversation. They may stop in the middle of a conversation and have no idea how to continue or they may repeat themselves. They may struggle with vocabulary, have problems finding the right word or call things by the wrong name (e.g., calling a watch a "hand clock").

Misplacing things and losing the ability to retrace steps: People with Alzheimer's may put things in unusual places and lose things and be unable to go back over their steps to find them again. Sometimes, they accuse others of stealing. This may occur more frequently over time.

Decreased or poor judgment: People with Alzheimer's may experience changes in judgment or decision-making. For example, they may use poor judgment when dealing with money, giving large amounts to telemarketers. They may pay less attention to grooming or keeping themselves clean

Withdrawal from work or social activities: People with Alzheimer's may start to remove themselves from hobbies, social activities, work projects or sports. They may have trouble keeping up with a favorite sports team or remembering how to complete a favorite hobby. They may also avoid being social because of the changes they have experienced.

Changes in mood and personality: The mood and personalities of people with Alzheimer's can change. They can become confused, suspicious, depressed, fearful or anxious. They may be easily upset at home, at work, with friends or in places where they are out of their comfort zones.[7,30]

1.8 Risk factors

Age : Increasing age is the greatest known risk factor for Alzheimer's disease. Alzheimer's is not a part of normal aging, but as you grow older the likelihood of developing Alzheimer's disease increases.

One study, for example, found that annually there were four new diagnoses per 1,000 people ages 65 to 74, 32 new diagnoses per 1,000 people ages 75 to 84, and 76 new diagnoses per 1,000 people age 85 and older.[31]

Family history and genetics : Your risk of developing Alzheimer's is somewhat higher if a first-degree relative — your parent or sibling — has the disease. Most genetic mechanisms of Alzheimer's among families remain largely unexplained, and the genetic factors are likely complex.[32]

One better understood genetic factor is a form of the apolipoprotein E gene (APOE). A variation of the gene, APOE e4, increases the risk of Alzheimer's disease. Approximately 25% to 30% of the population carries an APOE e4 allele, but not everyone with this variation of the gene develops the disease.

Scientists have identified rare changes (mutations) in three genes that virtually guarantee a person who inherits one of them will develop Alzheimer's. But these mutations account for less than 1% of people with Alzheimer's disease.[32]

Down syndrome : Many people with Down syndrome develop Alzheimer's disease. This is likely related to having three copies of chromosome 21 — and subsequently three copies of the gene for the protein that leads to the creation of beta-amyloid. Signs and symptoms of Alzheimer's tend to appear 10 to 20 years earlier in people with Down syndrome than they do for the general population.

Sex : There appears to be little difference in risk between men and women, but, overall, there are more women with the disease because they generally live longer than men.

Mild cognitive impairment : Mild cognitive impairment (MCI) is a decline in memory or other thinking skills that is greater than normal for a person's age, but the decline doesn't prevent a person from functioning in social or work environments.

People who have MCI have a significant risk of developing dementia. When the primary MCI deficit is memory, the condition is more likely to progress to dementia due to Alzheimer's disease. A diagnosis of MCI encourages a greater focus on healthy lifestyle changes, developing strategies to make up for memory loss and scheduling regular doctor appointments to monitor symptoms.[34]

Headtrauma : People who've had a severe head trauma have a greater risk of Alzheimer's disease. Several large studies found that in people age 50 years or older who had a traumatic brain injury (TBI), the risk of dementia and Alzheimer's disease increased. The risk increases in people with more-severe and multiple TBIs. Some studies indicate that the risk may be greatest within the first six months to two years after the TBI.[34]

Air pollution : Studies in animals have indicated that air pollution particulates can speed degeneration of the nervous system. And human studies have found that air pollution exposure — particularly from traffic exhaust and burning wood — is associated with greater dementia risk**.**

Excessive alcohol consumption : Drinking large amounts of alcohol has long been known to cause brain changes. Several large studies and reviews found that alcohol use disorders were linked to an increased risk of dementia, particularly early-onset dementia.

Poor sleep patterns :Research has shown that poor sleep patterns, such as difficulty falling asleep or staying asleep, are associated with an increased risk of Alzheimer's disease.[35]

Lifestyle and heart health : Research has shown that the same risk factors associated with heart disease may also increase the risk of Alzheimer's disease.

These include:

- Lack of exercise
- Obesity
- Smoking or exposure to secondhand smoke
- High blood pressure
- High cholesterol
- Poorly controlled type 2 diabetes

These factors can all be modified. Therefore, changing lifestyle habits can to some degree alter your risk. For example, regular exercise and a healthy low-fat diet rich in fruits and vegetables are associated with a decreased risk of developing Alzheimer's disease[32,34]

1.9 Diagnosis

An important part of diagnosing Alzheimer's disease includes being able to explain your symptoms, as well as perspective from a close family member or friend about symptoms and their impact on daily life. Additionally, a diagnosis of Alzheimer's disease is based on tests your doctor administers to assess memory and thinking skills.

Laboratory and imaging tests can rule out other potential causes or help the doctor better identify the disease causing dementia symptoms.But Alzheimer's disease is only diagnosed with complete certainty after death, when microscopic examination of the brain reveals the characteristic plaques and tangles.[35]

1.9.1 Tests

A diagnostic work-up would likely include the following tests:

Physical and neurological exam

Your doctor will perform a physical exam and likely assess overall neurological health by testing the following:

- Reflexes
- Muscle tone and strength
- Ability to get up from a chair and walk across the room
- Sense of sight and hearing
- Coordination
- Balance

1.9.2 Laboratory tests

Blood tests may help your doctor rule out other potential causes of memory loss and confusion, such as a thyroid disorder or vitamin deficiencies.[37]

Mental status and neuropsychological testing

Your doctor may give you a brief mental status test to assess memory and other thinking skills. Longer forms of neuropsychological testing may provide additional details about mental function compared with people of a similar age and education level. These tests can help establish a diagnosis and serve as a starting point to track the progression of symptoms in the future.[36]

Brain imaging

Images of the brain are now used chiefly to pinpoint visible abnormalities related to conditions other than Alzheimer's disease — such as strokes, trauma or tumors — that may cause cognitive change. New imaging applications — currently used primarily in major medical centers or in clinical trials — may enable doctors to detect specific brain changes caused by Alzheimer's.[37]

Imaging of brain structures include the following:

- **Magnetic resonance imaging (MRI).** MRI uses radio waves and a strong magnetic field to produce detailed images of the brain. While they may show brain shrinkage of brain regions associated with Alzheimer's disease, MRI scans also rule out other conditions. An MRI is generally preferred to a CT scan for the evaluation of dementia.
- **Computerized tomography (CT).** A CT scan, a specialized X-ray technology, produces cross-sectional images (slices) of your brain. It's usually used to rule out tumors, strokes and head injuries.[38]

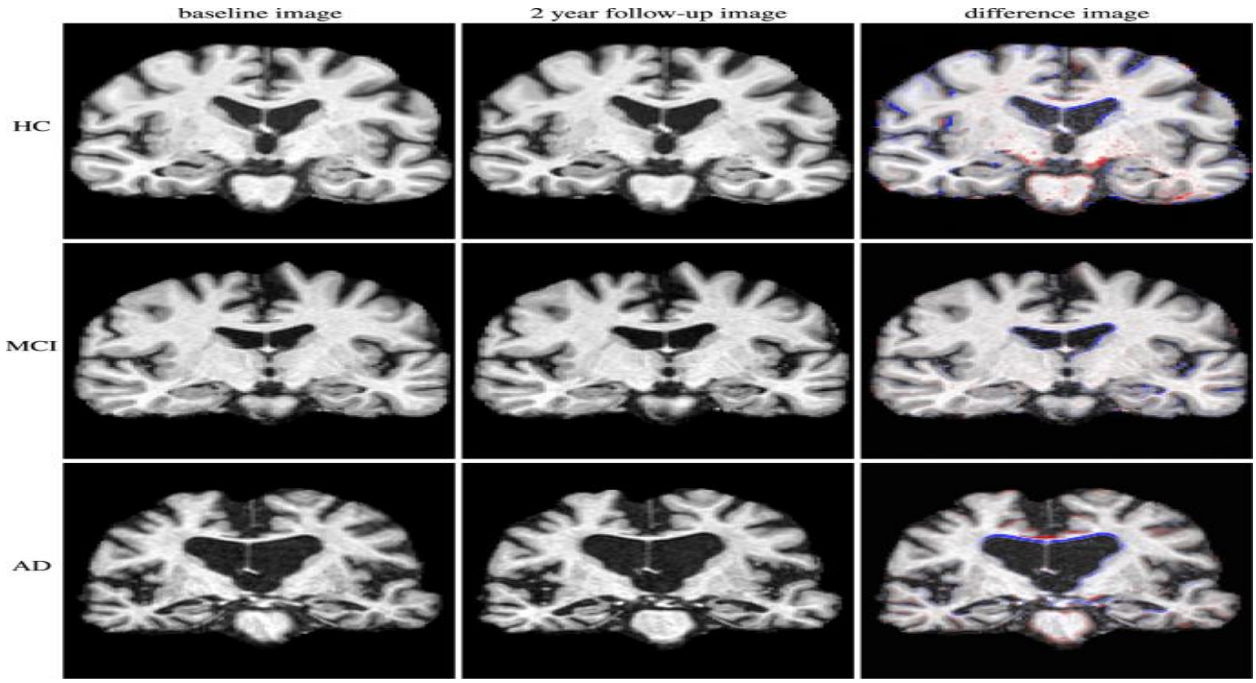


Figure 3: Structural brain imaging in Alzheimer's disease and mild cognitive impairment[38]

Imaging of disease processes can be performed with positron emission tomography (PET). During a PET scan, a low-level radioactive tracer is injected into the blood to reveal a particular feature in the brain. PET imaging may include the following:

- **Fluorodeoxyglucose (FDG) PET** scans show areas of the brain in which nutrients are poorly metabolized. Identifying patterns of degeneration — areas of low metabolism — can help distinguish between Alzheimer's disease and other types of dementia.
- **Amyloid PET imaging** can measure the burden of amyloid deposits in the brain. This imaging is primarily used in research but may be used if a person has unusual or very early onset of dementia symptoms.
- **Tau PET imaging,** which measures the burden of neurofibrillary tangles in the brain, is generally used in the research setting.

In special circumstances, such as rapidly progressive dementia, dementia with atypical features or early-onset dementia, other tests may be used to measure abnormal beta-amyloid and tau in the cerebrospinal fluid.[37,38]

1.9.3 Future diagnostic tests

Researchers are working to develop tests that can measure biological signs of disease processes in the brain.

These tests, including blood tests, may improve the accuracy of diagnoses and enable earlier diagnosis before the onset of symptoms. A blood test for Plasma Aβ is currently available and recently received certification in the U.S. by the Centers for Medicare & Medicaid Services to allow distribution on the market.

Genetic testing generally isn't recommended for a routine Alzheimer's disease evaluation. The exception is people who have a family history of early-onset Alzheimer's disease. Meeting with a

genetic counselor to discuss the risks and benefits of genetic testing is recommended before undergoing any tests.[39]

1.10 TREATMENT

1.10.1 **Non Pharmacologic Teatment**

Exercise

Physical exercise is an important component of non-pharmacologic therapy for sleep disturbances. In addition to the benefit of improving sleep, evidence from a randomized, controlled trial suggests that a home-based exercise program combined with behavioral management can reduce functional dependence, improve physical health and depression, and delay institutionalization among patients with Alzheimer's disease. A supervised exercise program in community-dwelling individuals is feasible. Most persons with dementia were able to walk for 30 or more minutes per day in one study[86]

Diet

Vitamin E. Although vitamin E doesn't prevent Alzheimer's, taking 2,000 international units daily may help delay the progression in people who already have mild to moderate disease. However, study results have been mixed, with only some showing modest benefits. Further research into the safety of 2,000 international units daily of vitamin E in a dementia population will be needed before it can be routinely recommended.

Supplements promoted for cognitive health can interact with medications you're taking for Alzheimer's disease or other health conditions. Work closely with your health care team to create a safe treatment plan with any prescriptions, over-the-counter medications or dietary supplements.[46,47]

Omega-3 fatty acids. Omega-3 fatty acids in fish or from supplements may lower the risk of developing dementia, but clinical studies have shown no benefit for treating Alzheimer's disease symptoms.[47]

Melatonin. This supplement of a hormone that regulates sleep is being studied to determine if it offers benefits managing sleep in people with dementia. But some research has indicated that melatonin may worsen mood in some people with dementia. More research is needed[49]

Reminiscence Therapy

Reminiscence therapy elicits recall of past events, activities, and memories through the use of tangible aids such as photographs, familiar items from the past, music and movies. While remembering recent memories (e.g. what one had for lunch) may prove difficult for individuals with dementia, long held memories of personal importance can remain easily accessible. Reminiscence therapy encourages participants to speak about past experiences therefore decreasing the demand on impaired cognitive abilities while encouraging those preserved abilities.[83]

Validation Therapy

Validation therapy is a form of "therapy for communicating with persons diagnosed as having Alzheimer's disease and related dementia. Focused on validating the personhood and emotions of a person with dementia, validation therapy posits that individuals with dementia present with confusion as a means to avoid stress, boredom, loneliness, and often as an escape from a reality. [84]It is then the responsibility of the facilitator (e.g. caregiver, clinician) to validate the feelings of the person with dementia rather than focus on the confusion as a means of comfort Validation therapy is found to alleviate stress, promote contentment, and decrease behavioral disturbances. Note that the benefit of validation therapy may be limited to those with mild-moderate form of the disease. This therapy focuses less on what is factually correct and more so on validating the person's feelings and emotions in their moment of confusion[85]

Cognitive symptoms (memory and thinking)

As Alzheimer's progresses, brain cells die and connections among cells are lost, causing cognitive symptoms to worsen. While these medications do not stop the damage Alzheimer's causes to brain cells, they may help lessen or stabilize symptoms for a limited time by affecting certain chemicals involved in carrying messages among and between the brain's nerve cells.[35]

The following medications are prescribed to treat symptoms related to memory and thinking

1.10.2 CHOLINESTERASE INHIBITORS

Neurotransmitter enhancement therapy with cholinesterase inhibitors (ChEIs) is a clinically proven approach for patients with mild-to moderate AD. Cholinesterase inhibitors increase cholinergic synaptic transmission by inhibiting acetylcholinesterase in the synaptic cleft, thereby decreasing the hydrolysis of acetylcholine released from the presynaptic neurons. These drugs result in small but measurable clinical benefit.[35]

CLASSIFICATION

Drug	Class	Dose (mg/day)	Frequency (times/day)	Absorption affected by food	Metabolism
Donepezil (Aricept)	Cholinesterase inhibitor	5-10†	1	No	CYP2D6 CYP3A4
Rivastigmine (Exelon)	Cholinesterase inhibitor	3-12	2	Yes	Non-hepatic
Galantamine (Reminyl; Reminyl PR)	Cholinesterase inhibitor	8-32	2 1 (PR)	Yes	CYP2D6 CYP3A4
Memantine (Ebixa)	NMDA-receptor antagonist	5-20	2 (one)	No	Non-hepatic

* PR denotes prolonged release, and NMDA N-methyl D-aspartate
† Donepezil 23 mg not available yet in Hong Kong

Table 1 :Symptomatic drug treatments for Alzheimer's[35]

Donepezil (Aricept)

Dosing and administration: Oral immediate release and oral disintegrating tablet Initial dose 5mg daily Increase after 1 month to maintenance dose 10mg daily Oral sustained release 23mg film-coated tablet

Mechanism of action: Reversible non-competitive acetylcholinesterase inhibitor

Pharmacokinetics/ metabolism: Protein binding: primarily proteinbound

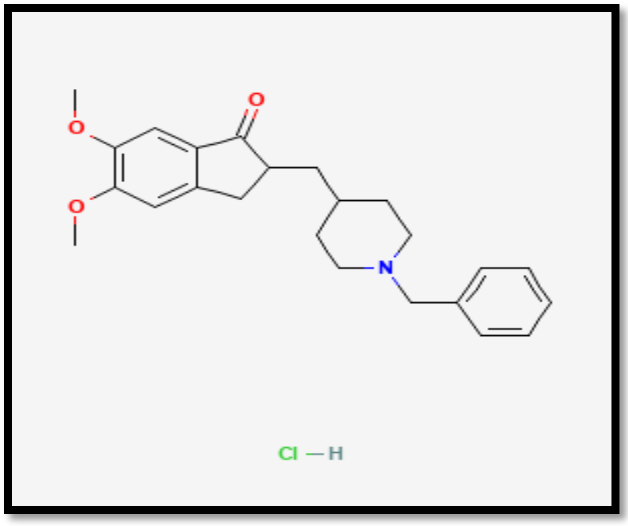


Figure 4: Donpezil [36]

Metabolism: hepatic, via CYP2D6 and CYP 3A4; 2 active and 2 inactive metabolites Half-life: 70 hours

Rivastigmine (Exelon)

Dosing and administration: Oral Initial dose 1.5mg BID with meals Increase by 3mg daily every 2 weeks to maintenance dose of 6mg BID Transdermal patch Initial dose 4.6mg patch to upper back daily Increase no sooner than 4 weeks to 9.5mg/day patch and then to maximum dose of 13.3mg/day patch Rotate patch site to reduce skin irritation

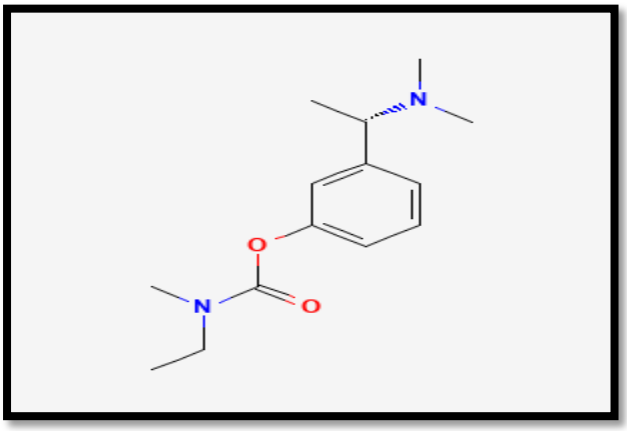


Figure 5: Rivastigmine [35]

Pharmacokinetics/ metabolism: Protein binding: 40% Metabolism: hydrolyzed in brain, then metabolite further processed in liver independent of CYP system then eliminated in urine Half life: 1.5 hours (oral), 3 hours (after patch removal), but clinical effect ~10 hours due to pseudoirreversible nature of inhibition[42]

Galantamine (Razadyne)

Dosing and administration: Oral immediate release Initial dose 4mg BID Increase by 8mg daily every 4 weeks to maintenance dose of 12mg BID Oral extended release Initial dose 8mg daily Increase by 8mg daily every 4 weeks to maintenance dose of 24mg daily Also available as oral solution[42]

Mechanism of action: Reversible, competitive acetylcholinesterase inhibitor and modulator of nicotinic acetylcholine receptor

Pharmacokinetics/ metabolism: Protein binding: low Metabolism: hepatic via CYP2D6 Half-life: 7 hours Adverse effects

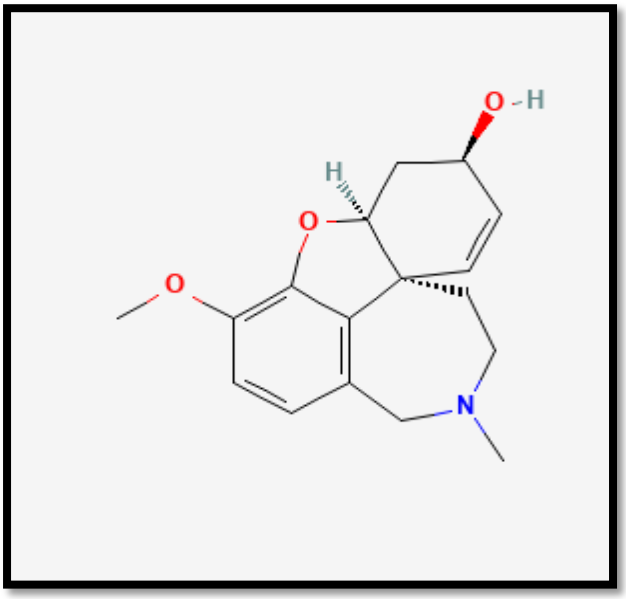


Figure 6: Galantamne[3]

Mechanism Of Action

The interaction of acetylcholinesterase with the substrate acetylcholine results in the breakdown, hydrolysis, and inactivation of acetylcholine and subsequent control of the amount of ACh at the synapse. AChE is a serine hydrolase that creates a tetrahedral intermediate through acid-base reactions with a catalytic triad (serine, histidine, acid residue).[36]

Histidine allows for the transference of a proton between the oxygen molecules in serine and ACh, thereby removing choline to form a new acylated serine. When the acylated serine is deacylated, the regeneration of free AChE begins. In this reaction, aspartate stabilizes the protonated histidine, which releases acetic acid and a new, free enzyme. The interaction between amino acid residues (tyrosine, phenylalanine, tryptophan) that make up a peripheral anionic site influences the conformational binding of ACh to that site.[34,35]

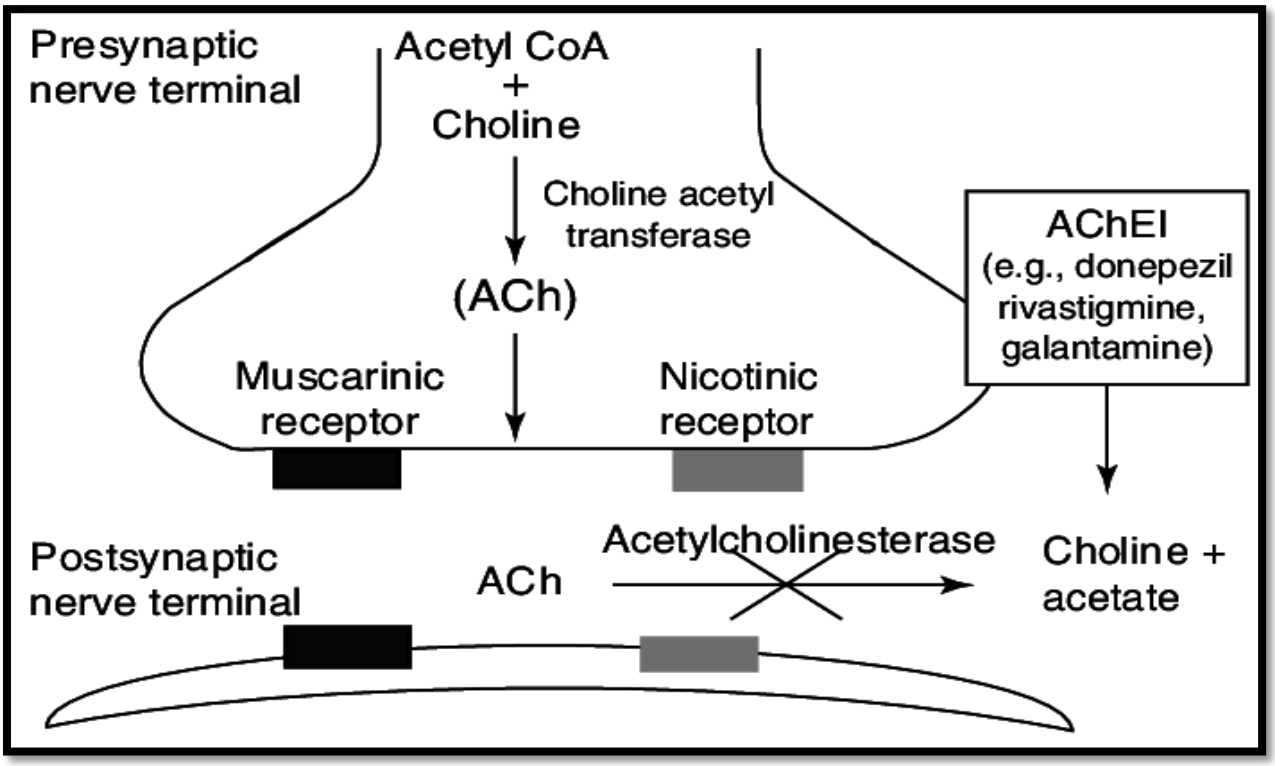


Figure 7: Acetylcholinesterase Inhibitors[35]

1.10.3 NMDA receptor antagonist

Memantine is an amantadine derivative that blocks the NMDA receptor channel in a manner similar to magnesium. During normal synaptic transmission, the NMDA receptor is transiently activated by the binding of glutamate and the coactivator glycine, resulting in phasic depolarisation of the neuron.

Pathological overactivation of the NMDA recep tor results in a persistent depolarisation of the neuron that relieves the magnesium block and allows an excessive calcium influx that may initiate synaptic μmol or dendritic damage, necrosis or apoptosis[44]

Memantine (Namenda)

Dosing and administration: Immediate release initial titration Week 1: 5mg daily Week 2: 5mg BID Week 3: 10mg qam, 5mg QHS Week 4 and after: 10mg BID Sustained release Namenda XR Initial dose: 7mg daily Increase weekly in increments of 7 mg to maintenance dose of 28mg daily Available in combination with donepezil as Namzaric

Pharmacokinetics/ metabolism: Protein binding: 45% Metabolism: almost 50% excreted unchanged in urine; remainder undergoes hepatic metabolism independent of CYP system Half-life: 60-80 hours

Mechanism of action: Non-competitive NMDA antagonist

Adverse effects: Generally well tolerated without consistent pattern of adverse effects; for example, package labeling includes both hypertension and hypotension, and constipation and diarrhea Rare hypersensitivity reactions have been reported[44,45]

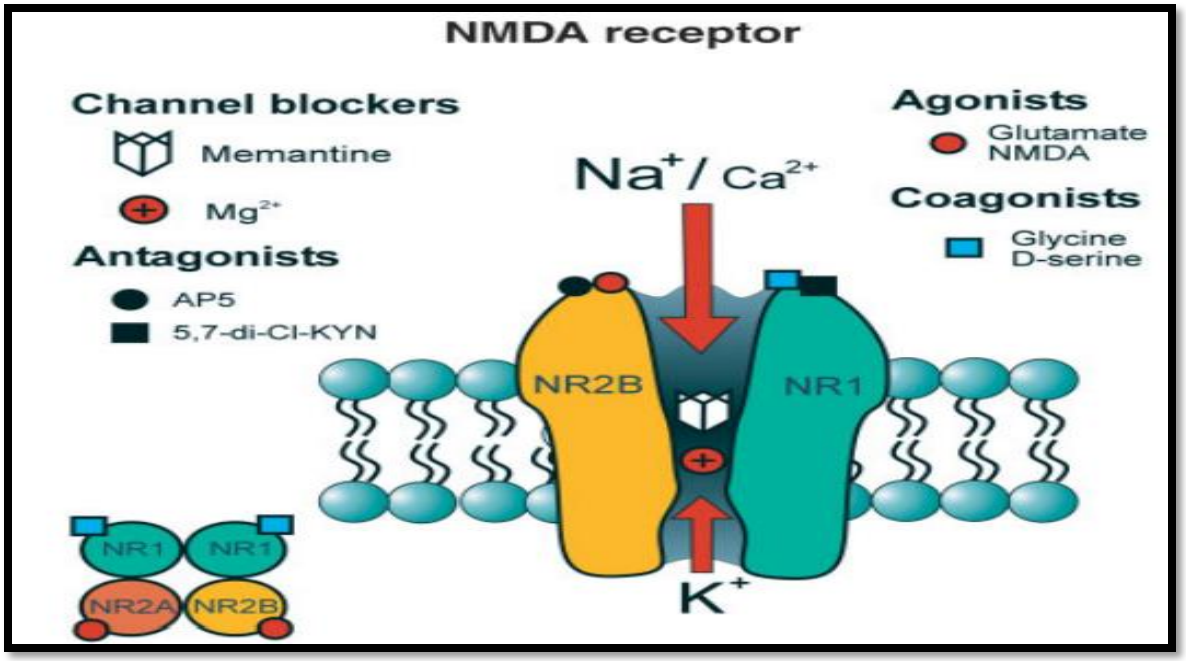


Figure 8 :Nmda Receptor[44]

The cognitive impairment of AD is closely related to synaptic plasticity, in which NMDAR plays a critical roleExcitatory glutamatergic neurotransmission via NMDAR is critical for synaptic plasticity and survival of neurons. However, excessive NMDAR activity causes excitotoxicity and promotes cell death, underlying a potential mechanism of neurodegeneration occurred in AD. The major factors that affect NMDAR signaling in AD include glutamate availability and the modulation of NMDAR channel functions

Some of the treatments that have been studied recently include:

- **Aducanumab-avwa (Aduhelm).** This human monoclonal antibody is the first therapy that targets the fundamental pathophysiology of the disease by reducing amyloid beta plaques in the brain. It is for use in those with early stages of Alzheimer's and with confirmed presence of amyloid pathology. It may cause swelling of bleeding in the brain[.50]

1.10.4 Adverse Effects Associated With Acetylcholinesterase

- Practitioners should always consider that the use of a cholinesterase inhibitor may contribute to a new or worsening clinical presentation. For example, such use has been associated with an increased risk of urinary incontinence, and donepezil has been associated with an increased use of hypnotics (presumably for the treatment of insomnia). The most common side effects of cholinesterase inhibitors are gastrointestinal (e.g., anorexia, nausea, vomiting, diarrhea).[34]
- Such effects are most likely to occur at the start of therapy or when the dose is increased. They are dose related and tend to be transient. In the clinical trials, gastrointestinal side effects appeared to be more common with rivastigmine than with the other cholinesterase inhibitors. Slower titration and ensuring rivastigmine is taken with food decreases the risk of gastrointestinal side effects.[36,37]
- The rivastigmine transdermal patch seems to be associated with less nausea and vomiting than the oral form.32 Weight loss did occur during the clinical trials of all 3 agents.. Dizziness has been reported with the use of all 3 agents. Syncope, although rare, has also occurred with these agents. Donepezil has been associated with sleep disturbances, vivid dreams or nightmares and hypnopompic hallucinations[35,36]

1.10.5 How To Overcome Adverse Effect Of Acetylcholinesterase

Medicinal plants are playing a significant role in the management of AD and memory deficit. The important traditional therapeutic methods are Ayurvedic, homeopathy, Unani and Sidha systems of medicine. Unani system of medicine offers traditionally a highly scientific health care therapy as a divine gift and as a result the global interest of the medical profession is focused on medicinal plants. Traditional system of medicine is fundamentally preventive, protective, nutritive and curative. Therefore, traditional medicines are safe and harmless which treat the patients with fewer or no side effects.

1.11 Neuroprotective Herbs for the Management of Alzheimer's Disease

Ashwagandha (*Withania somnifera*)

Ashwagandha, commonly called Indian ginseng or winter cherry, is one of the most prominent herbs prescribed as a brain rejuvenator for AD. It is prescribed to increase energy, improve overall health and longevity, and as a nerve tonic . Ashwagandha has been shown to possess antioxidant activity, free radical scavenging activity, as well as an ability to support a healthy immune system.[51] Ashwagandha contains several bioactive compounds of great interest, such as ergostane-type steroidal lactones, including withanolides A-Y, dehydrowithanolide-R, withasomniferin-A, withasomidienone, withasomniferols A-C, withaferin A, withanone, and others. Other constituents include the phytosterols sitoindosides VII-X and beta-sitosterol and alkaloids.[52]

Figure 9 :Ashwagandha[52]

Brahmi (*Bacopa monnieri*)

Brahmi, or Bacopa monnieri (Bm), is a perennial creeper medicinal plant found in the damp and marshy wetlands of Southern and Eastern India, Australia, Europe, Africa, Asia, and North and South America. In the Ayurvedic system of medicine, Bm is recommended for mental stress, memory loss, epilepsy, insomnia, and asthma .[53] The bioactive phytochemicals present in this plant include saponins, bacopasides III, IV, V, bacosides A and B, bacosaponins A, B, C, D, E, and F, alkaloids, sterols, betulic acid, polyphenols, and sulfhydryl compounds, which may be responsible for the neuroprotective roles of the plant.[56]

Figure: 10 Brahmi[56]

Cat's Claw (*Uncaria tomentosa*)

Cat's claw (CC) is a tropical vine with hooked thorns that resemble the claws of a cat and is mainly recommended for its potential role in the treatment of AD and pre-AD. It is found mainly in the Amazon rainforest and other areas of South and Central America.[54] This medicinal plant contains oxindole alkaloids, polyphenols (flavonoids, proanthocyanidins, and tannins), glycosides, pentacyclic alkaloids, and sterols . CC is known for its immune-modulating and anti-inflammatory effects and for its role as a free radical scavenger.[55]

Figure 11: Cat's Claw [55]

Ginkgo (***Ginkgo Biloba)***

Ginkgo biloba (Gb) has been in the spotlight primarily for its potential role in treating AD. Gb also appears promising as a therapeutic agent for several other chronic and acute forms of

diseases. The main pharmacologically active groups of compounds are flavonoids and terpenoids.[52] Almost all clinical studies use Gb extract that contains a combination of flavonoid glycosides, terpene lactones, and ginkgolic acids . Gb extract has shown beneficial effects in treating Alzheimer's, cardiovascular diseases, cancer, tinnitus, and other age-associated conditions. The suggested mechanisms of the Gb extract are its antioxidant effect, anti-platelet activating factor activity for vascular diseases, inhibition of β-amyloid peptide aggregation in AD, and decreased expression of peripheral benzodiazepine receptor for stress alleviation[57]

Figure 12: *Ginkgo Biloba*

Gotu Kola (*Centella asiatica*)

Considered both a nutraceutical and cogniceutical, Gotu kola (Gk) is a staple in Chinese, Indonesian, and Ayurvedic medicine . This medicinal plant is used to strengthen the brain, heal skin issues, and promote liver and kidney health. Gk is considered a rejuvenating herb for nerve and brain cells as it is believed to promote intelligence and improve memory[58]

Figure 13: Gotukola [58]

Saffron (*Crocus sativus*)

Saffron is a crimson-colored spice that is widely cultivated in Iran, India, and Greece. In addition to its usage in the textile and cosmetic industries, saffron is also recommended for its medicinal properties .[53] The major component of saffron is safranal, a carboxaldehyde. In vitro and in vivo studies show that the phytochemicals present in saffron possess antioxidant, anti-inflammatory, and anti-amyloidogenic properties[59]

Figure 14: Saffron[59]

Shankhpushpi (*Convolvulus pluricaulis*)

Shankhpushpi, or Convolvulus pluricaulis (Cp), is used for nerve regeneration and for improvement of memory . The major chemical components include triterpenoids, flavonol glycosides, anthocyanins, and steroids, which are responsible for Cp's nootropic and memory-enhancing properties .[54] Cholinergic and glutamatergic signaling can be enhanced by a group of nutraceuticals called racetams. Cp produces some similar effects to racetams. Cp modulates the body's production of adrenaline and cortisol . Cp is also recommended for mental stress and fatigue, anxiety, and insomnia[60]

Figure 15: **Shankhpushpi[60]**

Turmeric *(Curcuma longa)*

Turmeric is a flowering plant of the ginger family Zingiberaceae and is native to the Indian subcontinent and Southeast Asia. The bright yellow–orange color that this rhizome plant displays is mainly due to the polyphenolic compounds called curcuminoids.[52] Turmeric is anti-inflammatory, antiseptic, and antibacterial and has long been used to treat a wide variety of conditions including liver detoxification, to prevent infection and inflammation, to balance cholesterol levels, to treat allergies, to stimulate digestion, and to boost immunity [53]. The active constituents of turmeric are turmerone oil and watersoluble curcuminoids. Curcuminoids include curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and cyclocurcumin . Curcumin is the principal curcuminoid whose anti-inflammatory property is associated with reduced risk of AD . In vitro studies revealed curcumin's ability to block lipid peroxidation and neutralize reactive oxygen species, which was several times more potent than vitamin E[61]

Figure 16: curcumin [61]

1.12 Polyphenols

Natural biophenols are a wide group of molecules (over 8000 described so far) found only in the plant kingdom; their molecules display one or more aromatic rings carrying one or more hydroxyl groups; these molecules display remarkable antioxidant power and are produced as secondary metabolites by the plant for protection against the attack by bacteria, fungi, and insects (phytoalexins) .[62] Plant polyphenols include non-flavonoids or flavonoids; the latter are further divided into flavonols, flavononols, flavones, anthocyanins, procyanidins, phenolic acids, stilbenes, and tannins depending on the number of hydroxyls in the molecule and on the nature and the position of other substituents .

Plant polyphenols have been considered for their remarkable antioxidant properties; however, their effects go well beyond this property. In fact, plant polyphenols have been shown to possess beneficial effects against aggregation of peptides/proteins into amyloid assemblies, a process involved in several amyloid diseases, particularly T2DM, AD, and PD, thus reducing the load of intra- or extracellular deposits[66]

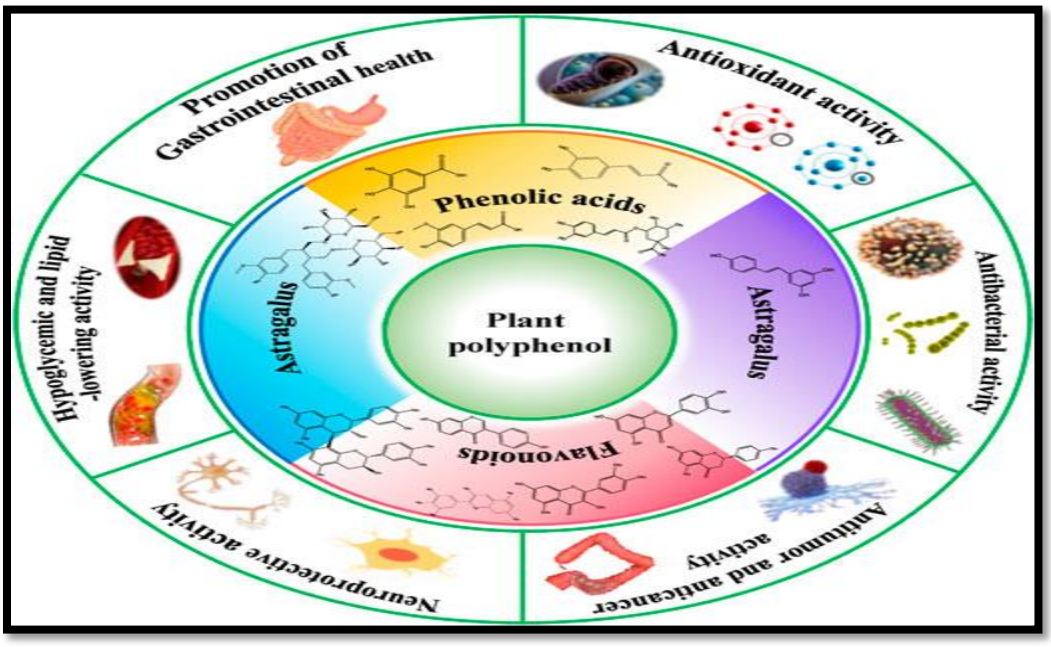


Figure 17 :Plant polyphenol's activities[62]

The beneficial effects of plant preparations and derivatives, including, in the case of olive, oil and leaf extracts, have already been known forthe last couple of centuries and have been scientifically investigated over the recent several decades; these researches have progressively led to a focus on the multi-target activity and health properties of plant polyphenols, including the anti-amyloid aggregation, antioxidant, antimicrobial, antihypertensive, hypoglycemic, and vasodilator effects. The antioxidant power has been shown to involve modulation of oxidative pathways , direct action on enzymes, proteins, receptors, and several types of signaling paths , as well as the interference with epigenetic modifications of chromatin . [64]

The clinical significance of the beneficial properties of plant polyphenols was first reported in 1950 , leading to the inclusion in the European Pharmacopoeia of the 80% alcoholic extract of olive leaves, containing oleuropein, HT, caffeic acid, tyrosol, apigenin, and verbascoside [65]. Biophenols can also be used to develop new drugs useful to combat chronic inflammatory conditions, the risk of thrombosis, CVD-related states such as atherosclerosis , cancer , also in combination with anti-cancer drugs , as well as to reduce amyloid deposition associated with T2DM and aging-related states such as neurodegeneration. Finally, the molecular scaffolds of plant polyphenols are also investigated to develop new molecules potentially exploitable in disease prevention and therapy[66]

A growing number of population surveys and clinical trials increasingly support the use of plant polyphenols, possibly in association with more specific drugs, to prevent and/or to treat several aging-associated pathologies. In conclusion, the rising interest in the nutraceutical/pharmacological exploitment of plant polyphenols or their molecular scaffolds holds promise that in the near future, the knowledge of the molecular/cellular determinants of the beneficial effects of these molecules, together with their pharmacokinetics and pharmacodynamics, will increase.[62]

It is also expected that results from more extended and convincing clinical trials will be reported, better focusing on benefits and, possibly, caveats associated with the use of these molecules or their chemical derivatives. Such increased information, provided it will further confirm the potential of plant polyphenols in the prevention/treatment of metabolic-, aging-, or lifestyle-associated pathologies presently without resolutive therapies, will allow a more general

use of these molecules as an important tool to prevent or to reduce the incidence of these increasingly widespread pathologies, ensuring safer aging[63]

1.12.1 Role Of Polyphenol's In Alzheimers Disease

Polyphenols are considered to have a protective effect against inflammatory mechanisms and as such have been linked to AD, as to many other chronic diseases including diabetes, metabolic syndrome, and atherosclerosis. Dietary intake of polyphenols is known to attenuate the progression of the disease by showing strong potential to tackle the alterations and reduce the risk of AD by reversing the cognitive deficits.

1.13 Introduction Of *Insilico*

Pharmacology over the past 100 years has had a rich tradition of scientists with the ability to form qualitative or semi-quantitative relations between molecular structure and activity *in cerebro*. To test these hypotheses they have consistently used traditional pharmacology tools such as *in vivo* and *in vitro* models. Increasingly over the last decade however we have seen that computational (*in silico*) methods have been developed and applied to pharmacology hypothesis development and testing.

These *in silico* methods include databases, quantitative structure-activity relationships, pharmacophores, homology models and other molecular modeling approaches, machine learning, data mining, network analysis tools and data analysis tools that use a computer. *In silico* methods are primarily used alongside the generation of *in vitro* data both to create the model and to test it.Such models have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization.

1.13 *Insilico* Drug Discovery Approach

The need for a rapid search for small molecules that may bind to targets of biological interest is of crucial importance in the drug discovery process. One way of achieving this search is by the *in silico* or the virtual screening method .

The term "*in silico*" is a modern word usually used to mean experimentation performed by computer and is related to the more commonly known biological terms *in vivo* and *in vitro*. *In silico* modeling bypass the traditional drug testing of compounds, synthesized and screened in multi-step time consuming processes and thus forms the basis of a new approach to drug discovery.[67] More specifically, it defines the use of information (protein crystallographic structures) in the creation of computational models (docked complexes) that can be used to make predictions, suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics .[68]

Figure-18*: Insilico* computational approach [70]

Molecular docking may be defined as an optimization problem, which would describe the best fit orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules .[69] This protein ligand interaction is the most studied due to its varied applications in drug discovery. The ligand is generally a small molecule, which interacts with the target

protein‘s binding sites. The protein-ligand interaction is comparable to the lock-and-key principle, in which the lock encodes the protein and the key is grouped with the ligand.[70]

The major driving force for binding appears to be hydrophobic interaction through several possible mutual conformations by which binding may occur. Therefore, computational approaches aid 'dock' small molecules into the structures of macromolecular targets and 'score' their potential complementarity to binding sites and are widely used in hit identification and lead optimization [71]. In simple terms, using bioinformatics tools, in silico techniques help in identifying potential drug target by: exploring the target structures for possible active sites, generating candidate molecules, docking these molecules with the target, ranking them according to their binding affinities, and further optimizing the molecules to improve binding characteristics .[72]

Thus, in modern drug designing, molecular docking is routinely used for understanding drug receptor interaction to predict the binding orientation of potential small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule . Further computational prediction of pharmacokinetic parameters like Absorption, Distribution, Metabolism and Excretion (ADME) and toxicity studies have become increasingly important in drug selection and promotion process and are promising tools for early screening of potential drug candidates .[73,74]

2 . PLANT PROFILE

Eugenia jambolana, or even jambul, is definitely an evergreen tree indigenous to exotic regions of India and parts of Asia.Jamun is one of the potential plants which are being used in treatment of several lifestyle diseases such as diabetes, cardiovascular diseases, age related macular degeneration and others in India for many decades.

Traditional Indian Medicines like Ayurveda and Unani prescribe Jamun for different health problems including diabetes, dental problems, digestive disorders, liver trouble and skin ailments. Keeping in view of the above factors, it was intended to adopt an overview on this highly medicinal plant.[76]

2.1 Vernacular Names

various common names of Jamun spoken in different regions of India and abroad are as follows:[77]

Language	Vernacular names
English	Black plum,jambol,black berry ,java plum ,
Sanskrit	Jambu
Tamil	Arugadam, kottaingram,neredam
Hindi	Jaman,jam,phalinda,bhojaman,kalajaman
Bengali	Jam,kalajam

Table 2: Plant Vernacular Names

2.2 Taxonomy

Kingdom	**Plantae**
Clade	Tracheophytes
Clade	Angiosperms
Clade	Rosids
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	S.cumini

Table 3 : Plant Taxonomy [76]

2.4 Distribution

The tree is found throughout India, Nepal, Myanmar, Sri Lanka, Indonesia, Pakistan, Bangladesh, Malaysia, Australia and other tropical regions of the world including South America and Madagascar from ancient time. It is very often cultivated for its edible fruits. The flowering starts from the month of March to May whereas the fruiting takes place during May to July every year.[79]

2.3 Morphological Character

It is an ever green tree that attains a height of 100 feet and can be 12 feet broad. Leaves 3 to 6 inch long and 2 to 3 inch broad. These are ovate shape and are shiny in texture. Flowers are light green to white in color. Fruits are ½ to 1 ½ inch long and oval in shape. When the fruit is raw it is green in color when it is ripe it becomes red or purple in color. The fruit contains seed that is about 1 to2 centimeter in length. Flowers are borne in early summers and fruits in autumn.[78]

Figure 19: *Eujenia Jambolan* A-Tree, B -Leaves [78]

2.5 Chemical Constituents

Edible pulp : protein, fat, carbohydrate. Minerals: Ca, Mg, P, Fe, Na, K; Vitamins: vitamin A; thiamine; riboflavin; nicotinic acid; vitamin C; and folic acid. Sugar: Glucose and Fructose. Acid: Malic acid (mainly) oxalic acid, Gallic acid and tannins. Waxy component: essential oil; triterpenehydroxy acid; oleanolic acid.

Flowers: Three triterpenoids; acetyl oleanolic acid, Eugenia-triterpenoid A, and Eugeniatriterpenoid B. Flavonoids: isoquercitrin, quercitin, kaempferol and myricetin.

Seeds: Protein, Calcium, Tannin, ellagic acid, Gallic acid; a glycoside (jamboline); essential oils; Oleic, myristic, linoleic, stearic, palmitic, vernolic, lauric, sterculic and malvalic acids. [76,78]

Leaves: Polyphenols, Protein. Tannins, Essential oil. Flavonoids,

Bark: Betulinic acid, Beta-sitosterol, Friedelin, Tannins, Gallic acid, Ellagic acid and myricetin, Eugenin and fatty acid ester.[80]

2.6 Nutritional Properties

Jamun is a good source of minerals, which contains calcium, potassium, magnesium, phosphorus, sodium and vitamin C. Energy value per 100 g of edible fruit is 62 Kcal. It also contains protein, carbohydrates, carotene and folic acid. Jamun is used in making beverages, jellies, jam, squash, wine, vinegar and pickles[76,79]

2.7 Pharmacologic Activities

Jamun has been reported to be used in numerous Complementary and Alternative Medicines and different medical systems of India. Before the discovery of insulin, it was a frontline antidiabetic medication even in Europe. [80]

In ethnomedicobotanical literature the following pharmacological actions are mentioned; Antidiabetic, Hypolipidemic, Antibilious, Digestive, Carminative, Appetizer, Stomachic, General tonic and liver tonic, Antidiarrheal, Astringent to bowel, Enriches the blood, Strengthens the teeth and gums, Antiscorbutic, Diuretic, Anti-inflammatory, Anthelmintic and Antimicrbial activity.[81]

2.8 Traditional Uses

Leaves:

- Dysentery
- Ash of leaves is used for strengthening the teeth
- Rapid healing of injuries.[82

Fruits: The fruit is sour, acrid, sweet;

- Useful astringent in bilious diarrhoea;
- Good gargle for sorehroat;
- Good lotion for ringworm in the head.
- It removes bad smell from the mouth.
- The vinegar from the fruit is useful in diseases of the spleen.

Bark: Bark is acrid and sweet

- Digestive, astringent to the bowels
- Anthelmintic
- Good for sore throat
- Bronchitis, asthma,
- Thirst, biliousness, dysentery,
- Blood impurities and ulcers.[7]

Seed: The seed is acrid and sweet

- Astringent to the bowels,
- Good for diabetes
- Allays thirst in diabetes

3. LITERATURE REVIEW

1. **Mark W. Bondi**, **et al(2017)** had reviewed on past, present and future on alzheimers. We review this lineage of work beginning with Alzheimer's own writings and drawings, then jump to the modern era beginning in the 1970s and early 1980s and provide a sampling of neuropsychological and other contextual work from each ensuing decade. During the 1980s our field began its foundational studies of profiling the neuropsychological deficits associated with AD and its differentiation from other dementias (e.g., cortical vs. subcortical dementias). The 1990s continued these efforts and began to identify the specific cognitive mechanisms affected by various neuropathologic substrates. The 2000s ushered in a focus on the study of prodromal stages of neurodegenerative disease before the full-blown dementia syndrome (i.e., mild cognitive impairment). The current decade has seen the rise of imaging and other biomarkers to characterize preclinical disease before the development of significant cognitive decline. Finally, we suggest future directions and predictions for dementia-related research and potential therapeutic interventions.
2. **Armand S. Schachter ,et al(2000),** Alzheimer's disease is one of the most devastating brain disorders of elderly humans. It is an undertreated and under-recognized disease that is becoming a major public health problem. The last decade has witnessed a steadily increasing effort directed at discovering the etiology of the disease and developing pharmacological treatment. Recent developments include improved clinical diagnostic guidelines and improved treatment of both cognitive disturbance and behavioral problems. Future directions in the research and treatment of patients with Alzheimer's disease include: applying functional brain imaging techniques in early diagnosis and evaluation of treatment efficacy; development of new classes of medications working on different neurotransmitter systems (cholinergic, glutamatergic, etc), both for the treatment of the cognitive deficit and the treatment of the behavioral disturbances; and developing preventive methods (amyloid p-peptide immunizations and inhibitors of β-secretase and γ-secretase).

3. **Dugu M**, **et al(2003),** Elderly persons are at increased risk for developing dementia, and this risk increases with age. It is important to understand the following points: (a). how to diagnose dementia; (b). the etiology of the most common dementias (including Alzheimer s disease, ischemic vascular dementia, and diffuse Lewy body dementia); (c). some medical conditions which could contribute to symptoms of dementia; (d). the pathophysiology of Alzheimer s disease; and (e). management problems faced by caregivers for dementia patients. This review aims to educate clinicians to focus on caregivers issues and the need for long-term planning.

4. **C.-Y. Chiao RN ,et al(2015)** had reviewed on Dementia is an irreversible illness. The caregiver is expected to assume increased responsibility as the condition of the person with dementia declines. It is important to explore the factors constituting caregiver burden on the informal caregivers of people with dementia. A systematic review of the four databases, including PubMed, PsycINFO, CINAHL and the Cochrane Library, was carried out to access relevant articles published between 2003 and 2012. Twenty-one articles met the inclusion criteria of this study.

5. **Martin Prince ,et al(2013)** this study they provide a systematic review of the global literature on the prevalence of dementia (1980–2009) and metaanalysis to estimate the prevalence and numbers of those affected, aged 60 years in 21 Global Burden of Disease regions The evidence base on the prevalence of dementia is expanding rapidly, particularly in countries with low and middle incomes. A reappraisal of global prevalence and numbers is due, given the significant implications for social and public policy and planning. Future projections of numbers of people with dementia may be modified substantially by preventive interventions (lowering incidence), improvements in treatment and care (prolonging survival), and disease-modifying interventions (preventing or slowing progression). All countries need to commission nationally representative surveys that are repeated regularly to monitor trends

6. **ChristopherPatterson, et al(2007)** This review identifies and quantifies general (ie, nongenetic) risk factors for all-cause dementia, Alzheimer's disease, and vascular dementia specifically.

7. **Alzheimer's Association (2016)** This report had reviewed on the public health impact of Alzheimer's disease, including incidence and prevalence, mortality rates, costs of care, and the overall impact on caregivers and society. It also examines in detail the financial impact of Alzheimer's on families, including annual costs to families and the difficult decisions families must often make to pay those costs.

8. **Constantine G. Lyketsos ,et al(2011)** had reviewed on Neuropsychiatric symptoms on Alzheimer's disease and related dementias. Once thought to emerge primarily in people with late-stage disease, these symptoms are currently known to manifest commonly in very early disease and in prodromal phases, such as mild cognitive impairment. Despite decades of research, reliable treatments for dementia-associated NPS have not been found, and those that are in widespread use present notable risks for people using these medications. An Alzheimer's Association Research Roundtable was convened in the spring of 2010 to review what is known about NPS in Alzheimer's disease, to discuss classification and underlying neuropathogenesis and vulnerabilities, and to formulate recommendations for new approaches to tailored therapeutics

9. **Francis T Hane**, **et al(2017)** had reviewed on The field of Alzheimer's disease (AD) research has grown exponentially over the past few decades, especially since the isolation and identification of amyloid-β from postmortem examination of the brains of AD patients. Recently, the Journal of Alzheimer's Disease (JAD) put forth approximately 300 research reports which were deemed to be the most influential research reports in the field of AD since 2010.

10. **Sneham Tiwari ,et al(2019)** had reviwed on Currently, 47 million people live with dementia globally, and it is estimated to increase more than threefold (~131 million) by 2050. Alzheimer's disease (AD) is one of the major causative factors to induce

progressive dementia. AD is a neurodegenerative disease, and its pathogenesis has been attributed to extracellular aggregates of amyloid β (Aβ) plaques and intracellular neurofibrillary tangles made of hyperphosphorylated τ-protein in cortical and limbic areas of the human brain. It is characterized by memory loss and progressive neurocognitive dysfunion

11. **Saikat Sen**, **et al(2013)** had done a work on to determine the total phenolic and total flavonoid contents, and to evaluate the antioxidant potential of different leaf extracts of Meyna spinosa Roxb. ex Link, a traditional medicinal plant of India. he results indicated a direct correlation between the antioxidant activity and the polyphenolic content of the extracts, which may the foremost contributors to the antioxidant activity of the plant. The present study confirmed that the methanol extract of Meyna spinosa leaves is a potential source of natural antioxidants.

12. **Archana Raju**, **et al(2021)** had done a work on Polyphenols for their potential involvement in the prevention of various chronic diseases as well as for their antimicrobial potential. The crude extracts of arecanut have been reported to have antiinfective properties. We aimed to explore the endosperm of Areca catechu (arecanut) for the extraction of polyphenol components and to study the antituberculosis activity of these polyphenol against Mycobacterium tuberculosis H37Rv. Polyphenols have been studied for their potential involvement in the prevention of various chronic diseases as well as for their antimicrobial potential. The crude extracts of arecanut have been reported to have antiinfective properties. We aimed to explore the endosperm of Areca catechu (arecanut) for the extraction of polyphenol components and to study the antituberculosis activity of these polyphenol against Mycobacterium tuberculosis H37Rv.

13. **R Madaan, et.al(2011)** had done a work on Actaea spicata Linn. (Ranunculaceae) has been traditionally used for the treatment of various ailments such as rheumatism, inflammation, nerve diseases, lumbago, scrofula and chorea, but no systematic phytochemical and pharmacological work has ever been carried out on this potential plant. Preliminary phytochemical screening showed presence of phenols and flavonoids

in A. spicata. Thus, the present investigation was undertaken to estimate total phenols and flavonoids in methanol extract of A. spicata roots, and its ethyl acetate fraction.

14. **Salim Ahammed ,et al(2021)** had done a work on Vanda roxburghii has been used in traditional medicine to treat nervous system disorders including Alzheimer's disease (AD). We reported earlier a high acetylcholinesterase inhibitory and antioxidant activity in the chloroform fraction of this plant. Therefore, this study was designed to explore the compounds with acetylcholinesterase inhibitory and antioxidant activities from the chloroform fraction of Vanda roxburghii. Phytochemical investigation led to the isolation for the first time of a fatty acid ester: methyl linoleate (1), and three phenolics: syringaldehyde (2), vanillin (3), and dihydroconiferyl dihydro-p-coumarate (4) along with the previously reported compound gigantol (5). Among the isolates, vanillin (3) and dihydroconiferyl dihydro-p-coumarate (4) were found to significantly inhibit the activity of acetylcholinesterase, scavenge the free radicals, exhibit the reducing power and total antioxidant activity, and effectively reduce the peroxidation of lipid.

15. **Daniel ZaBuski ,et al (2016)** had done a work on Neurodegenerative diseases .The aim of this work focused on the screening of the natural inhibitors of AChE and BuChE and antioxidants in Eleutherococcus species. HPTLC screening confirmed the presence of inhibitors in extracts. All extracts exhibited anti-DPPH∗ activity and single antioxidants have been identified. To the best of our knowledge, no information was available on this activity of compounds in Eleutherococcus. These studies provide a biochemical basis for the regulation of AChE and BuChE and encourage us to continue isolation of active compounds.

16. **Julie Gregory, et al(2021)** had reviewed on background ogf Alzheimer’s disease (AD) is a multifactorial, progressive, neurodegenerative disease that is characterized by memory loss, personality changes, and a decline in cognitive function. While the exact cause of AD is still unclear, recent studies point to lifestyle, diet, environmental, and genetic factors as contributors to disease progression. The pharmaceutical approaches developed

to date do not alter disease progression. More than two hundred promising drug candidates have failed clinical trials in the past decade, suggesting that the disease and its causes may be highly complex. Medicinal plants and herbal remedies are now gaining more interest as complementary and alternative interventions and are a valuable source for developing drug candidates for AD. Indeed, several scientific studies have described the use of various medicinal plants and their principal phytochemicals for the treatment of AD

17. **Manuela Leri ,et al(2020)**had reviewed on The increasing extension in life expectancy of human beings in developed countries is accompanied by a progressively greater rate of degenerative diseases associated with lifestyle and aging, most of which are still waiting for effective, not merely symptomatic, therapies. Accordingly, at present, the recommendations aimed at reducing the prevalence of these conditions in the population are limited to a safer lifestyle including physical/mental exercise, a reduced caloric intake, and a proper diet in a convivial environment. The claimed health benefits of the Mediterranean and Asian diets have been confirmed in many clinical trials and epidemiological surveys. These diets are characterized by several features, including low meat consumption, the intake of oils instead of fats as lipid sources, moderate amounts ofred wine, and significant amounts of fresh fruit and vegetables. In particular, the latter have attracted popular and scientific attention for their content, though in reduced amounts, of a number of molecules increasingly investigated for their healthy properties. Among the latter, plant polyphenols have raised remarkable interest in the scientific community; in fact, several clinical trials have confirmed that many health benefits of the Mediterranean/Asian diets can be traced back to the presence of significant amounts of these molecules, even though, in some cases, contradictory results have been reported, which highlights the need for further investigation. In light of the results of these trials, recent research has sought to provide information on the biochemical, molecular, epigenetic, and cell biology modifications by plant polyphenols in cell, organismal, animal, and human models of cancer, metabolic, and neurodegenerative pathologies, notably Alzheimer's and Parkinson disease.

18. **Umesh Chandra Dash ,et al(2017)** had done a work on Geophila repens (L.) I. M. Johnst. (Rubiaceae), a small, creeping, perennial herb, is claimed to have memory-enhancing property. The goal of this study was to assess its antioxidant and anticholinesterase activity and conduct a rapid bioautographic enzyme assay for screening acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition of G. repens extracts. METHODS: Antioxidant activity of G. repens extracts was assessed by performing 1,1-diphenyl-2- picrylhydrazyl (DPPH), nitric oxide (NO), superoxide (SOD), hydroxyl (OH) and total antioxidant capacity (TAC) assays. Anticholinesterase activity was investigated by quantifying the AChE and BChE inhibitory activities of chloroform (CGR), ethyl acetate (EGR) and methanol (MGR) extract fractions from G. repens leaves. A rapid high-performance thin-layer chromatography (HPTLC) bioautographic method for the detection of AChE and BChE inhibition was performed. RESULTS: Among all extract fractions, EGR exhibited the highest half maximal inhibitory concentration (IC50) in DPPH, SOD, NO, OH and TAC assays, with IC50 of (38.33 ± 3.21), (45.14 ± 1.78), (59.81 ± 1.32), (39.45 ± 0.79) and (43.76 ± 0.81) µg/mL respectively. EGR displayed competitive, reversible inhibition of AChE and BChE activities with IC50 of (68.63 ± 0.45) and (59.45 ± 0.45) µg/mL, respectively. Total phenolic and flavonoids contents of EGR were found to be 360.42 mg gallic acid equivalents and 257.31 mg quercetin equivalents per gram of extract. Phytoconstituents of the EGR extract that were inhibitors of cholinesterase produced white spots on the yellow background of HPTLC plates in the bioautographic test

19. **Md. Abul Hasnat, et al(2013)** had done a work on the acetylcholinesterase inhibition and in vitro and in vivo antioxidant activities of Ganoderma lucidum grown on germinated brown rice (GLBR) were evaluated. In antioxidant assays in vitro, GLBR was found to have strong metal chelating activity, DPPH, ABTS, hydroxyl and superoxide radical scavenging activity. Cell-based antioxidant methods were used, including lipid peroxidation on brain homogenate and AAPH-induced erythrocyte haemolysis. In antioxidant assays in vivo, mice were administered with GLBR and this significantly enhanced the activities of antioxidant enzymes in the mice sera, livers and brains. The amount of total phenolic and flavonoid compounds were 43.14 mg GAE/g and 13.36 mg

CE/g dry mass, respectively. GLBR also exhibited acetylcholinesterase inhibitory activity. In addition, HPLC analyses of GLBR extract revealed the presence of different phenolic compounds. These findings demonstrate the remarkable potential of GLBR extract as valuable source of antioxidants which exhibit interesting acetylcholinesterase inhibitory activity.

20. **Tiyyaba Furqan, et al(2020)** had done a work to find the molecular interactions of some of the cannabinoid constituents of cannabis with acetylcholinesterase (AChE). Molecular docking and LogP determination were performed to predict the AChE inhibitory effect and lipophilicity. AChE enzyme activity was measured in the blood of cannabis addicted human subjects. Further, genetic predisposition to cannabis addiction was investigated by association analysis of cannabinoid receptor 1 (CNR1) single nucleotide polymorphism (SNP) rs806368 and ACHE rs17228602 using restriction fragment length polymorphism (RFLP) method. All the understudied cannabis constituents showed promising binding affinities with AChE and are lipophilic in nature. The AChE activity was observed to be indifferent in cannabis addicted and non-addicted healthy controls. There was no significant association with CNR1 SNP rs806368 and ACHE rs17228602. The study concludes that in silico prediction for individual biomolecules of cannabis is different from in vivo physiological action in human subjects when all are present together. However, for a deeper mechanistic insight into these interactions and association, multi-population studies are suggested. Further studies to explore the inhibitory potential of different cannabis constituents for intended AChE inhibitor-based drug are warranted.

21. **Lucas S. Frota, et al(2021)** had done a work on Ouratea fieldingiana is a native medicinal plant from Northeastern Brazil and many biological properties are due to the phenolic constituents. The objective of this work was performing the characterization of O. fieldingiana leaf constituents to correlate with antioxidant and anticholinesterase activities by in vitro and in silico studies and thus contribute to find new agents against Alzheimer's disease. The high-performance liquid chromatography revealed the presence

of the flavonoids rutin, isoquercitrin, kaempferol-3-O-rutinoside, quercetin, apigenin and amentoflavone. The antioxidant activities by the (2,2-diphenyl-1-picrylhydrazyl) (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methodologies, showed good results with half maximal inhibitory concentration (IC50) values ranging from 5.63 to 11.47 µg mL-1 and 2.72 to 23.71 µg mL-1, respectively. Acetylcholinesterase inhibition assay pointed out the flavone apigenin with best activity. Computational studies evaluated the interaction of flavonoids with the enzyme acetylcholinesterase co-crystallized with the galantamine, used as standard. All flavonoids exhibited binding energy greater than that of galantamine, but only apigenin showed strong interaction with the active site of the enzyme and other bind probably to different allosteric centers. Then, O. fieldingiana extract and flavonoids with good anti-radical activity and presenting a broad-spectrum action against acetylcholinesterase (AChE) enzyme ought to be tested in clinical studies to discover new neuro-therapeutic candidates.

22. **Ibrahim H. Borai, et al(2017)** had done a work on Alzheimer's disease (AD) is a grave and prevailing neurodegenerative disease, characterized by slow and progressive neurodegeneration in different brain regions. Aluminum (Al) is a potent and widely distributed neurotoxic metal, implicated in the neuropathogenesis of AD. This study aimed to evaluate the possible neurorestorative potential of Vitis vinifera Leaves Polyphenolic (VLP) extract in alleviating aluminum chloride (AlCl3)-induced neurotoxicity in male rats. AlCl3 neurotoxicity induced a significant decrease in brain/serum acetylcholine (ACh) contents and serum dopamine (DA) levels, along with a significant increment of brain/serum acetylcholinesterase (AChE) activities. In addition, Al treatment resulted in significantly decreased serum levels of both total antioxidant capacity (TAC) and brain-derived neurotrophic factor (BDNF), and significantly increased serum levels of both interleukin-6 (IL-6) and total homocysteine (tHcy), as compared to control. Behavioral alterations, assessed by the T-maze test, showed impaired cognitive function. Furthermore, AD-brains revealed an increase in DNA fragmentation as evidenced by comet assay. AlCl3 induction also caused histopathological alterations in AD-brain. Treatment of AD-rats with VLP extract (100

mg/kg body weight/day) improved neurobehavioral changes, as evidenced by the improvement in brain function, as well as, modulation of most biochemical markers, and confirmed by T-maze test, the histopathological study of the brain and comet assay. The current work indicates that the VLP extract has neuroprotective, antioxidative, anti-inflammatory, and anti-amnesic activities against AlCl3-induced cerebral damages and neurocognitive dysfunction

23. **Chistiane Mendes, et al (2015)** Feitosa had done a work on the anticholinesterase and antioxidant activities of Eugenia dysenterica DC. (O. Berg. (Myrtaceae) essential oils from leaves (EOED). EOED were obtained by hydrodistillation using a Clevenger-type apparatus and the products were analyzed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID). The main constituents of EOED were caryophyllene oxide (66.3%), isoledene (3.9%), 1,3,8-p-menthatriene (3.5%), mustakone (3.46%), βphellandrene (1.7%), and selin-11-en-4-α-ol (1.7%). The antioxidant assay was performed based on the formation of thiobarbituric acid reactive substances (TBARS), hydroxyl radical, and nitric oxide production. By performing the Ellman assay, it was observed that EOED was able to inhibit the enzyme g.ml-1μacetylcholinesterase (AChE) with an IC50 = 0.92 promising better value when compared with the g.ml-1μdrug rivastigmine (IC50 = 1.87), used in the treatment of Alzheimer's disease. The caryophyllene g.ml-1μoxide (the main compound) was tested after purification on the AChE with an IC50 = 0.31 . Caryophyllene oxide (the majority compound) was tested on the AChE and presented the IC50 = 0.31 g.ml-1μ g.ml-1μ. At concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 , it was found out that EOED prevented lipid peroxidation inhibiting amount of TBARS formed in a similar manner to ascorbic acid. In addition, there was a reduction in the production of hydroxyl radical as well as the production of nitric oxide. To the best of our knowledge, this is the first report on compounds from this species that have activity for potentially preventing neurodegenerative disorders.

24. **Ibrahim I. Mahmoud, et al(2001)** done a work on Two acylated flavonol glycosides and 15 known polyphenols have been isolated and identified from the leaves of Eugenca

jambolana Lam. The structures of the new compounds were identified as 3-O-(400-O-acetyl)-a-l-rhamnopyranoside of mearnsetin (myricetin 40 -methyl ether) and myricetin 3-O-(400-O-acetyl-200-O-galloyl)-a-l-rhamnopyranoside. The complete structure elucidation of all isolated metabolites based on chemical and spectroscopic methods of analysis (UV, 1D and 2D NMR) as well as negative ESI–MS with and without CID in-source fragmentation

25. **Lujuan Xing, et al(2019)** they did a work on Tea, leaf, or bud from the plant Camellia sinensis, make up some of the beverages popularly consumed in different parts of the world as green tea, oolong tea, or black tea. More particularly, as a nonfermented tea, green tea has gained more renown because of the significant health benefits assigned to its rich content in polyphenols. As a main constituent, green tea polyphenols were documented for their antioxidant, anti-inflammation, anticancer, anticardiovascular, antimicrobial, antihyperglycemic, and antiobesity properties. Recent reports demonstrate that green tea may exert a positive effect on the reduction of medical chronic conditions such as cardiovascular disease, cancer, Alzheimer's disease, Parkinson's disease, and diabetes. The health benefits of green teas, in particular EGCG, are widely investigated, and these effects are known to be primarily associated with the structure and compositions of its polyphenols. This Review focuses on the diverse constituents of green tea polyphenols and their molecular mechanisms from the perspective of their potential therapeutic function. Recent advances of green tea polyphenols on their bioavailability, bioaccessibility, and microbiota were also summarized in this article. Dietary supplementation with green tea represents an attractive alternative toward promoting human health.

26. **Anil J. Johnson ,et al(2010)** the word for this project had done on Melicope lunu-ankenda (Gaertn.) T.G. Hartley is used in Indian traditional medicine for fever, improving complexion and as a tonic. Previous studies have isolated fungicidal, antifeedant, antiinflammatory and immunomodulatory compounds from Melicope lunu-ankenda. This study is aimed at the isolation and biological activity screening of potential molecules from the volatile oils and extracts of Melicope lunu-ankenda in the light of

traditional applications. Materials and methods: Volatile oil of Melicope lunu-ankenda leaves was isolated by hydrodistillation, characterized by GC–FID, GC–MS, LRI determination, Co-GC and database searches. Major chromenetype compounds in Melicope lunu-ankenda leaf oil, evodione and leptonol, were isolated by preparative TLC and characterized by UV–Vis, IR, 1H-, 13C-, 13C-DEPT NMR and EIMS. They were also isolated from the petroleum ether and acetone extracts of the leaves of Melicope lunu-ankenda by column chromatography in petroleum ether–ethyl acetate. Their contents in leaf oil, leaf and inflorescence extracts were estimated by HPTLC. Antipyretic (Baker's yeast-induced fever test), analgesic (acetic acid-induced writhing, tail immersion assays), anti-inflammatory (carrageenan-induced paw edema) and in vitro antioxidant (DPPH radical, superoxide radical scavenging) activities of evodione and leptonol were tested. Results and conclusions: Gas chromatographic analyses found 50.7% monoterpene hydrocarbons, 0.4% oxygenated monoterpenes, 3.2% sesquiterpene hydrocarbons, 0.7% oxygenated sesquiterpenes and 43.7% chromene-type compounds in Melicope lunu-ankenda leaf oil, with evodione (20.2%) and leptonol (22.5%) as its two major constituents. HPTLC estimations in the petroleum ether, acetone extracts (leaf, inflorescence) and leaf oil found evodione 1.0% (dr. wt., leaf), 1.1% (inflorescence), 0.04% (fr. wt. leaves, leaf oil), and leptonol 0.3% (leaf), 0.3% (inflorescence) and 0.04% (leaf oil). Leptonol (200 mg/kg) showed good antipyretic activity. DPPH radical scavenging assay found moderate activity for leptonol (68.7%, 500 M), whereas evodione showed near-zero activity. A very similar trend was found in superoxide radical scavenging activity of leptonol (64.5%) and evodione (10.3%), both at 100 g/ml. Evodione and leptonol showed moderate analgesic activities in acetic acid-induced writhing and tail immersion assays. Moderate anti-inflammatory activity was found for both evodione (59.4%) and leptonol (49.0%) at 100 mg/kg.

27. **Balwinder Singh, et al(2018)** in this paper they reviewed on Jambolan is a rich source of bioactive phenolic compounds that have many potential health benefits. Phenolic acids, flavonoids (mainly anthocyanins, flavonols, flavanols and flavanonols) and tannins are the major phenolic compounds present in different parts of jambolan plant. Jambolan fruit skin mainly contains anthocyanins (such as delphinidin, petunidin, malvidin in

glycosylated forms), while the pulp is primarily rich in phenolic acids (such as gallic acid and ellagic acid) and tannins (mostly ellagitannins). Moreover, many other compounds have been reported to be present in jambolan fruit. Apart from fruit skin and pulp, jambolan leaves contain flavonoids such as quercetin, myricetin and flavonol glycosides, while seeds are known to contain ellagic acid, gallic acid and quercetin. Health-promoting activities of phenolic compounds present in jambolan reported in the literature are functioning as anti-inflammatory, anti-allergic, antihyperglycaemic, anticancer, cardioprotective, radioprotective, antibacterial, chemopreventive and antioxidant agents.

28. **S. Shyamala Gowri, et al(2010)** in this paper they had done a work on Phytochemical investigation was carried out on the crude methanol and aqueous extracts of the leaves of Syzygium cumini (L.) (MYRTACEAE). The antimicrobial activity of the extract was tested against standard strains and clinical isolates of some bacteria using the disc diffusion method. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of The gram negative bacteria such as Salmonella enteritidis, Salmonella typhi, Salmonella typhi A, Salmonella paratyphi A, Salmonella paratyphi B, Pseudomonas aeruginosa and Escherichia coli. gram positive bacteria are Bacillus subtilis, and Staphylococcus aureus. The results showed that the methanol extracts was more potent than the aqueous extracts. Key words: Syzygium cumini (L.) (Myrtaceae) Leaves Extracts Phytochemical Screening ,Antibacterial Activity.

29. **Upasna Balyan, et al(2019)** they had done a work on a hybrid process consisting of extraction and microfiltration was proposed in this study for producing purified, clear and stable aqueous phenolic extract from jamun (Syzygium cumini (L.) Skeels) leaf. Response surface methodology was successfully used for optimization of extraction conditions. Pseudo-first order kinetic model successfully described the extraction of total polyphenols from jamun leaves, with the activation energy determined as 9.5 kJ/mol based on the Arrhenius model. The kinetic constants were used to study the kinetic and thermodynamic compensations of extraction of TPC from jamun leaves. Applying the

statistical criterion, the kinetic and thermodynamic compensations were concluded to be real and the extraction process was controlled by entropy. A total of ten phenolic compounds including six phenolic acids (tannic acid, gallic acid, ellagic acid, caffeic acid, ferulic acid and p-coumaric acid) and four flavonoids (catechin, epicatechin, quercetin and myricetin 3-O-rhamnoside) were identified and quantified in jamun leaf extract obtained under optimum extraction conditions. The selection of appropriate membrane in the microfiltration step was a critical aspect. To observe the effect of membrane pore size on the permeate flux and permeate quality, leaf extracts were then microfiltered using four different microfiltration membranes (0.1, 0.22, 0.45, and 0.8 μm) under batch concentration mode. The flux decline was successfully described by the Hermia's cake filtration model. The stability of clarified extract was investigated at 4 °C for 45 days. The 0.45 μm microfiltration membrane was suggested for the clarification of jamun leaf extract in order to achieve high flux, polyphenol recovery, extract purity and improved storage stability

30. **D. Avila-Pe na, et al(2007)** they did a work on Syzygium jambos (L.) Alston (Myrtaceae) (syn Eugenia jambos) is a widespread medicinal plant traditionally used in sub-Saharan Africa to treat several diseases. The analgesic potential of leaf hydro-alcoholic extracts was assessed in rats. Hot plate and formalin tests were used to estimate cutaneous nociception whereas measurements of forelimb grip force were done to assess muscular nociception under normal and inflammatory conditions. In the hot plate test, Syzygium jambos extract produced a significant increase in the withdrawal response latencies in a dose-dependant manner (10–300 mg/kg i.p.) and with a maximal effect (analgesic efficacy) similar to that of morphine. The extract (100–300 mg/kg i.p.) significantly reduced pain scores in all the phases of the formalin test with an analgesic efficacy higher than that shown by diclofenac. Although the extract (300 mg/kg) did not alter grip force in intact rats, it reversed the reduction in grip force induced by bilateral injection carrageenan in the forelimb triceps. This analgesic effect of the extract on muscle hyperalgesia was not antagonized, but enhanced, by naloxone. Thus, the Syzygium jambos extract has remarkable analgesic effects on both cutaneous and deep muscle pain that is not mediated by opioid receptors

31. **Guilherme Ferreira de Oliveira, et al(2007)** had done a workThe antimicrobial activity of Syzygium cumini leaves extract, known as "jambolão", was evaluated. The crude hydroalcoholic extract was active against Candida krusei (inhibition zone of 14.7 ± 0.3 mm and MIC = 70 µg/ mL), and against multi-resistant strains of Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus.

32. **Zhi Ping Ruan, et al(2008)** the work was done on The antioxidant activity of Syzygium cumini leaf extracts was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power (FRAP) assays. The methanolic extract and its four water, ethyl acetate, chloroform, and n-hexane fractions were prepared and subjected to antioxidant evaluation. The results showed that the ethyl acetate fraction had stronger antioxidant activity than the other ones. HPLC data indicated that S. cumini leaf extracts contained phenolic compounds, such as ferulic acid and catechin, responsible for their antioxidant activity. A significant linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of leaf extracts supported this observation

33. **Jian-Guo Song, etal(2021)** this work is based on the typical HPLC-UV-MS profiles and characteristic 1 H NMR signals, twelve new phloroglucinol-derived lipids (1− 12), featuring a long linear aliphatic side chain, together with three known ones (13–15) were isolated from the ethanol extract of the leaves of Syzygium cumini. Their structures were elucidated on the basis of extensive NMR spectroscopic analyses and mass spectrometric data. Compounds 1–5 characterize an enolizable β,β'-tricarbonyl motif with a cyclohexa-3,5-dien-1-one core that is hitherto undescribed in phloroglucinol-derived lipids. Compounds 4 and 10–12 are novel phloroglucinol-derived lipids containing an uncommon methylene interrupted trans double bond in their polyunsaturated aliphatic side chains. A polyketide biogenetic pathway for those phloroglucinol-derived lipids was also proposed. In addition, the isolates were evaluated for their neuroprotective activities against oxygen-glucose deprivation and re-oxygenation (OGD/R)-induced Neuro-2a cell

injury. Notably, compounds 1, 5, and 10–12 significantly improved viability of Neuro-2a cells after OGD/R damage.

34. **Malek Ennaifer ,et al(2018)** this research had done on Tunisia, Pelargonium graveolens is widely consumed as a food aromatizing hydrosol. Recent studies have shown the potential of plant solvent-free extracts as food and pharmaceutical natural additives. Accordingly, in this study, we investigate the phenolic content, the volatile fractions of green P. graveolens extracts such as infusion and decoction, and we evaluate their biological activities. The total phenolic content of the infusion (27.05 mg GAE/ gDM) is significantly different from that of decoction (31.2 mg GAE/gDM). The GC-MS analysis identified about twenty volatile components in both extracts. The DPPH inhibition and the b-carotene bleaching tests of the infusion and the decoction had considerable results. Besides, infusion and decoction exhibited a relatively high anti-acetyl-cholinesterase activity and a considerable antimicrobial activity against S. aureus, among three tested pathogenic bacteria.

35. **Xiao Dan Zhang, et al(2013)** The aim of this study was to investigate chemical constituents of the leaves of Acanthopanax henryi, and their antioxidant, acetyl cholinesterase inhibitory activities. Caffeoyl quinic acid derivates and flavonoids were obtained from A. henry, through column chromatography technologies, and the content of major constituents was determined by the HPLC–UV method. Anti-oxidant activity of the isolated metabolites was evaluated by free radical scavenging (DPPH, ABTS radicals) and superoxide anion scavenging. The results showed that di-caffeoyl quinic acid derivates had stronger antioxidant activity than positive controls (ascorbic acid, trolox and allopurinol). Acetyl cholinesterase inhibitory activity was estimated on the constituents, among which, quercetin, 4-caffeoyl-quinic acid and 4,5-caffeoyl quinic acid were found to have strong acetyl cholinesterase inhibitory activity with IC50 values ranging from 62.6 to 121.9 lM. The present study showed that some of the tested constituents from the leaves of A. henryi exhibit strong antioxidant and acetyl cholinesterase inhibitory effects. This suggest that the leaves of A. henryi can be used as

a new natural complementary source of acetyl cholinesterase inhibitors and anti-oxidant agents, thus being a promising potential complementary source against Alzheimer's disease

36. **Sergey U. Savelev, et al(2003**) this work is done on extracts of Salvia (sage) species have been reported to have cholinergic activities relevant to the treatment of Alzheimer's disease. A lack of information on the inhibition of the enzyme butyrylcholinesterase, also considered to be a target in the treatment of the disease, prompted this in vitro investigation of the essential oils of S. fruticosa, S. lavandulaefolia, S. officinalis and S. officinalis var. purpurea for anti-butyrylcholinesterase activity. Dose-dependent inhibition of human cholinesterases by the extracts and constituents was determined using the method of Ellman. A time dependent increase in the inhibition of butyrylcholinesterase by the oils of S. fruticosa and S. officinalis var. purpurea was evident. IC50 values decreased from 0.15 ± 0.007 and 0.14 ± 0.007 mg/mL after 5 min to 0.035 ± 0.016 and 0.06 ± 0.018 mg/mL after 90 min incubation time respectively. The slow onset of inhibition of butyrylcholinesterase was also shown by individual constituents, such as 3- carene and β-pinene. Analyses of the chemical composition of the oils and anti-butyrylcholinesterase activity of their constituents revealed that none of the compounds tested would account for the total activity of the oils and that synergy is likely

37. **Taiwo Olayemi Elufioye ,et al(2017)** had done a work on Spondias mombin has been used in traditional medicine for the management of several diseases, including memory loss. This study aimed to evaluate the cholinesterase inhibitory activity of the methanol extract of the leaves and its derived fractions, as well as carry out detailed phytochemical investigations leading to the isolation and characterization of bioactive compounds from the plant. The acetyl cholinesterase (AChE) and butyryl cholinesterase (BUChE) inhibitory activities were evaluated by colorimetric and thin-layer chromatography bioautographic assay techniques. The ethyl acetate fraction was most active against both enzymes, with percentage inhibition of 58.10 ± 1.08% and 52.66 ± 1.34% against AChE and BUChE, respectively. Three compounds, namely, botulin, campesterol and phytol,

with IC50 of 0.88 µg/mL (AChE), 4.67 µg/mL (BuChE); 1.89 µg/mL (AChE), 4.08 µg/mL (BuChE) and 12.51 µg/mL (AChE), 23.89 µg/mL (BuChE), respectively, were isolated from the supernatant of the ethyl acetate fraction. The isolated cholinesterase inhibitory compounds correlate with the known memory-enhancing property of the plant and thus support one of its uses in ethnomedicine

38. **Jae Sue Choi ,et al(2014**) this research work is based on part of our ongoing isolation of cholinesterase (ChE) inhibitors from natural marine sources, the bioactivity of the ethanolic extracts from 12 Korean seaweeds were screened for their inhibitory activities against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and total reactive oxygen species (ROS) generation. Eisenia bicyclis exhibited promising inhibitory properties against AChE, BChE and total ROS with inhibition percentages (%) of 68.01 ± 1.37, 95.72 ± 3.80, and 73.20 ± 1.82 at concentrations of 25 lg/mL, respectively. Among the different solvent–soluble fractions obtained from the ethanolic extract, the ethyl acetate (EtOAc) fraction was found to cause the most potent scavenging, or inhibitory activities, against 2,2-diphenyl-1-picrylhydrazyl (DPPH), peroxynitrite (ONOO-) and total ROS with the respective IC50 values of 2.48 ± 0.01, 8.70 ± 0.06, and 0.81 ± 0.03 lg/mL.. 974-B showed strong scavenging/or inhibitory potential against DPPH, ONOO-, total ROS, AChE, and BChE with the respective IC50 values of 0.86 ± 0.02, 1.80 ± 0.01, 6.45 ± 0.04, 1.95 ± 0.01, and 3.26 ± 0.08 lM, respectively. These results indicate that the potential of E. bicyclis and its phlorotannin for use in the development of therapeutic or preventive agents of Alzheimer's disease mainly through ChE inhibition and additional antioxidant capacities.

39. **Dicson Sheeja Malar, et al(2016)** this studyhas done a work on Grewia tiliaefolia Vahl. (Tiliaceae) is a sub-tropical plant used as an indigenous medicine in India. However, its efficacy has not been evaluated against Alzheimer's disease. The objective of this study is to evaluate cholinesterase inhibitory, anti-aggregation and neuroprotective activity of G. tiliaefolia. Grewia tiliaefolia leaves were collected from Eastern Ghats region, India, and subjected to successive extraction (petroleum ether, chloroform, ethyl acetate, methanol and water). The extracts were subjected to in vitro antioxidant,

anticholinesterase and anti-aggregation assays. The active methanol extract (MEGT) was separated using column chromatography. LC-MS analysis was done and the obtained compounds were docked against acetylcholinesterase (AChE) enzyme to identify the active component. Results: Antioxidant assays demonstrated that the MEGT showed significant free radical scavenging activity at the IC50 value of 71.5 ± 1.12 lg/mL. MEGT also exhibited significant dual cholinesterase inhibition with IC50 value of 64.26 ± 2.56 and 54 ± 0.7 lg/mL for acetyl and butyrylcholinesterase (BChE), respectively. Also, MEGT showed significant anti-aggregation activity by preventing the oligomerization of Ab25–35. Further, In silico analysis revealed that vitexin binds effectively with AChE through strong hydrogen bonding. These results were further confirmed by evaluating the activity of vitexin in vitro, which showed dual cholinesterase inhibition with IC50 value of 15.21 ± 0.41 and 19.75 ± 0.16 lM for acetyl and butyrlcholinesterase, respectively. Grewia tiliaefolia can be considered as a promising therapeutic agent for the treatment of AD.

40. **T. Vivek Kumar, et al(2016)** had done a work on the antibacterial and antioxidant potential of Tiliacora racemosa leaf extracts in various solvents (methanolic, hexane, chloroform and ethyl acetate) was determined. Additionally, the presence of bisbenzylisoquinoline alkaloids in the plant prompted us to evaluate the nootropic activity of the methanolic extract in mice. Further, we seek to verify the nootropic effect by examining the anticholinesterase inhibition potential of the methanolic extract. The leaf extracts in various solvents were evaluated for their antibacterial and antioxidant activity by agar diffusion technique and a, a-diphenyl-b-picrylhydrazyl (DPPH) free radical scavenging method, respectively. The ex vivo acetylcholine esterase inhibitory activity of the methanolic extract was carried out by Ellman's method in male Wistar rats. The nootropic capacity of the methanolic extract was examined in Swiss albino mice by utilizing the diazepam induced acute amnesic model. The chloroform/n-hexane and ethyl acetate fraction showed promising antioxidant and antibacterial (Gram positive and Gram negative bacteria) property, respectively. The methanolic extract was able to diminish the amnesic effect induced by diazepam (1 mg/kg i.p.) in mice. The extract also showed significant acetyl cholinesterase inhibition in rats. The findings prove that the

memory enhancing capability is due to increased acetyl choline level at the nerve endings. The strong antioxidant nature and potential nootropic activity shown by the extract suggests its future usage in the treatment of neurodegenerative disorders such as dementia and Alzheimer.

41. **Sandeep Kumar Singh, et al(2019)** Alzheimer's disease (AD) is the most common progressive human neurodegenerative disorder affecting elderly population worldwide. Hence, prevention of AD has been a priority of AD research worldwide. Based on understanding of disease mechanism, different therapeutic strategies involving synthetic and herbal approaches are being used against AD. Among the herbal extract, Ginkgo biloba extract (GBE) is one of the most investigated herbal remedy for cognitive disorders and Alzheimer's disease (AD). Standardized extract of Ginkgo biloba is a popular dietary supplement taken by the elderly population to improve memory and agerelated loss of cognitive function. Nevertheless, its efficacy in the prevention and treatment of dementia remains controversial. Specifically, the added effects of GBE in subjects already receiving "conventional" anti-dementia treatments have been to date very scarcely investigated. This review summarizes recent advancements in our understanding of the potential use of Ginkgo biloba extract in the prevention of AD including its antioxidant property. A better understanding of the mechanisms of action of GBE against AD will be important for designing therapeutic strategies, for basic understanding of the underlying neurodegenerative processes, and for a better understanding of the effectiveness and complexity of this herbal medicine

42. **Franziska Pohl ,et al(2018)** had done a research on neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and Huntington's disease, present a major health issue and financial burden for health care systems around the world. The impact of these diseases will further increase over the next decades due to increasing life expectancies. No cure is currently available for the treatment of these conditions; only drugs, which merely alleviate the symptoms. Oxidative stress has long been associated with neurodegeneration, whether as a cause or as part of the downstream results caused by other factors. Thus, the use of antioxidants to counter cellular oxidative stress within

the nervous system has been suggested as a potential treatment option for neurological disorders. Over the last decade, significant research has focused on the potential use of natural antioxidants to target oxidative stress. However, clinical trial results have lacked success for the treatment of patients with neurological disorders. The knowledge that natural extracts show other positive molecular activities in addition to antioxidant activity, however, has led to further research of natural extracts for their potential use as prevention or treatment/management of neurodegenerative diseases. This review will cover several in vitro and in vivo research studies, as well as clinical trials, and highlight the potential of natural antioxidants.

43. **Tomoharu Kuboyama, et al (2014)** Neurodegenerative diseases commonly induce irreversible destruction of central nervous system (CNS) neuronal networks, resulting in permanent functional impairments. Effective medications against neurodegenerative diseases are currently lacking. Ashwagandha (roots of Withania somnifera DUNAL) is used in traditional Indian medicine (Ayurveda) for general debility, consumption, nervous exhaustion, insomnia, and loss of memory. In this review, we summarize various effects and mechanisms of Ashwagandha extracts and related compounds on in vitro and in vivo models of neurodegenerative diseases such as Alzheimer's disease and spinal cord injury

44. **Minsook Ye ,et al(2015)** this research work is done on Alzheimer's disease (AD) is the most common cause of dementia. This disease is a progressive and irreversible brain disorder accompanied with severe learning and memory impairment. This study investigated whether treatment with standardized Lycii Fructus Extract (LFE) would improve the cognitive function and the pathological features of AD in 3xTg-AD mice. Ethnopharmacological relevance: Lycii Fructus is a fruit of Lycium chinense Miller and widely distributed in East Asia and has been used traditionally for anti-aging purposes. Materials and methods: The cognitive function of 3xTg-AD mice was assessed using the Morris water maze test. The levels of the amyloid beta deposits and NeuN in the hippocampus were evaluated with immunohistochemistry. Brain neurotrophic derived factor (BDNF) and tyrosine kinase B (TrkB) expressions were examined by western blot

analysis. Results: LFE treatment significantly ameliorated learning and memory deficits in AD mice, as shown by increased time spent in the target zone during probe tests. In addition, LFE significantly decreased Aβ deposits, increased NeuN-positive cells, and upregulated the expression of BDNF and TrkB in the 3xTg AD mice

45. **G.C. Roma´n, et al(2019)** the paper had reviewed on the mechanisms of action of the dietary components of the Mediterranean diet are reviewed in prevention of cardiovascular disease, stroke, age-associated cognitive decline and Alzheimer disease. A companion article provides a comprehensive review of extravirgin olive oil. The benefits of consumption of long-chain v-3 fatty acids are described. Fresh fish provides eicosapentaenoic acid while a-linolenic acid is found in canola and soybean oils, purslane and nuts. These v-3 fatty acids interact metabolically with v-6 fatty acids mainly linoleic acid from corn oil, sunflower oil and peanut oil. Diets rich in v-6 fatty acids inhibit the formation of healthier v-3 fatty acids. The deleterious effects on lipid metabolism of excessive intake of carbohydrates, in particular high-fructose corn syrup and artificial sweeteners, are explained. The critical role of the v-3 fatty acid docosahexaenoic acid in the developing and aging brain and in Alzheimer disease is addressed. Nutritional epidemiology studies, prospective population-based surveys, and clinical trials confirm the salutary effects of fish consumption on prevention of coronary artery disease, stroke and dementia. Recent recommendations on fish consumption by pregnant women and potential mercury toxicity are reviewed. The polyphenols and flavonoids of plant origin play a critical role in the Mediterranean diet, because of their antioxidant and anti-inflammatory properties of benefit in type-2 diabetes mellitus, cardiovascular disease, stroke and cancer prevention. Polyphenols from fruits and vegetables modulate tau hyperphosphorylation and beta amyloid aggregation in animal models of Alzheimer disease. From the public health viewpoint worldwide the daily consumption of fruits and vegetables has become the main tool for prevention of cardiovascular disease

46. **Manuela Leri ,et al(2020)** The increasing extension in life expectancy of human beings in developed countries is accompanied by a progressively greater rate of degenerative diseases associated with lifestyle and aging, most of which are still waiting for effective,

not merely symptomatic, therapies. Accordingly, at present, the recommendations aimed at reducing the prevalence of these conditions in the population are limited to a safer lifestyle including physical/mental exercise, a reduced caloric intake, and a proper diet in a convivial environment. The claimed health benefits of the Mediterranean and Asian diets have been confirmed in many clinical trials and epidemiological surveys. These diets are characterized by several features, including low meat consumption, the intake of oils instead of fats as lipid sources, moderate amounts ofred wine, and significant amounts of fresh fruit and vegetables. In particular, the latter have attracted popular and scientific attention for their content, though in reduced amounts, of a number of molecules increasingly investigated for their healthy properties. Among the latter, plant polyphenols have raised remarkable interest in the scientific community; in fact, several clinical trials have confirmed that many health benefits of the Mediterranean/Asian diets can be traced back to the presence of significant amounts of these molecules, even though, in some cases, contradictory results have been reported, which highlights the need for further investigation. In light of the results of these trials, recent research has sought to provide information on the biochemical, molecular, epigenetic, and cell biology modifications by plant polyphenols in cell, organismal, animal, and human models of cancer, metabolic, and neurodegenerative pathologies, notably Alzheimer's and Parkinson disease. The findings reported in the last decade are starting to help to decipher the complex relations between plant polyphenols and cell homeostatic systems including metabolic and redox equilibrium, proteostasis, and the inflammatory response, establishing an increasingly solid molecular basis for the healthy effects of these molecules. Taken together, the data currently available, though still incomplete, are providing a rationale for the possible use of natural polyphenols, or their molecular scaffolds, as nutraceuticals to contrast aging and to combat many associated pathologies.

4. AIM AND OBJECTIVE

Aim

The aim of this study was to perform the polyphenolic extraction of *Eugenia jambolana* leaves and to evaluate the antioxidant activity , anti cholinesterase activity on Aluminium trichloride induced Alzheimers in rat model and *In-silico* Docking approaches to evaluate the anticholinesterase activity . For achieving the aim, we have the following objectives.

Objectives

1. Identify ,collection and authentication of *Eugenia jambolana* leaf
2. To prepare extract of *Eugenia jambolana* leaf and its preliminary phytochemical evaluation
3. Estimation of total phenolic content by Folin Ciocalteu Method
4. To investigate the anti- alzhimers avtivity of *Eugenia jambolana* leaf extract in aluminium induced rat alzheimers model
5. *In-silico* Docking Approaches to evaluate the anticholinesterase activity

The Alzheimers activity is evaluated by estimating

- Antioxidant activity- DPPH activity on HPTLC
- *In-vivo* test: Morris water maze test
- *Ex-vivo* test in rat: Biochemical analysis for estimation of brain AChE activity

5. PLAN OF WORK

- ***PHASE I***

  - Literature survey
  - Plant collection & authentication
  - Approval from IAEC committee

- ***PHASE II***

  - Prepare plant extraction and evaporation
  - Preliminary Phytochemical analysis
  - Estimation of total phenolic content
  - Lead and Target identification

- ***PHASE III***

  In-silico studies

  - *In-silico* molecular docking study
  - ADMET prediction study

- Evaluation of antioxidant activity
- Pharmacological screening

  In-vivo study

  - Estimation of learning and memory by Morris water maze test
  - Estimation of anticholinesterase.
- Documentation of results.

6 .MATERIALS AND METHODS

6.1 Materials

The following softwares are used to perform the *In-silico* evaluations such as Drug likeness, ADMET prediction and Molecular docking

S.NO	SOFTWARE NAME	MODE	PURPOSE
1.	PubChem	Online/Free	Collection of Ligands, Drug likeness
2.	RCSB database	Online/Free	Collection of Proteins
3.	pkCSM	Online/Free	ADMET prediction
4.	SPDB viewer	Offline/Free	Protein preparation
5.	BIOVIA Discovery Studio	Offline/Free	a. Ligands collection and clustering b. To study protein ligand interaction
6.	PyRx	Offline/Free	Docking process
7.	PyMOL	Offline/Free	Building the protein ligand complex

Table 4: List of Softwares

6.2 Methods

6.2.1 Ligand identification

The three-dimensional structure andtwo-dimensional structure which have listed on Table 6 was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).In order to find its physicochemical properties such as molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity and topological polar surface area and its properties were compared with Lipinski's rule of five for the evaluation of Drug likeness.

6.2.2 Target identification

The three-dimensional structure of the selected proteins were retrieved from RCSB database (https://www.rcsb.org/) in Protein Data Bank (PDB) format. Drug Target is a biomolecule which is involved in signaling or metabolic pathways that are specific to a disease process. The 2 selected Proteins are of *Homo sapiens* origin. AChE,BuChE which are listed on Table no 8

List of Protein

S.NO	PROTEIN	PDB ID
1.	AChE	4PQE
2.	BuChE	4TPK

Table 5: Targeted Proteins

List of ligand

S.no	Phytoconstituents	Pub Chem CID
1.	Methyl gallate	7428
2.	Myricitrin	5281673
3.	Nilocitin	14021529
4.	Rivastigmine	77991

Table 6: list of ligands

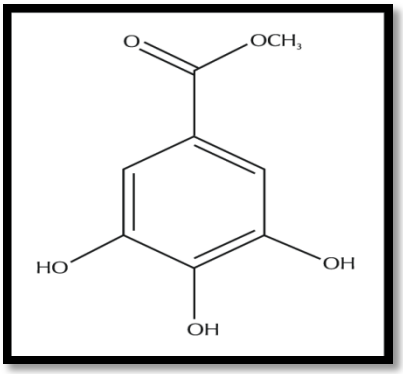
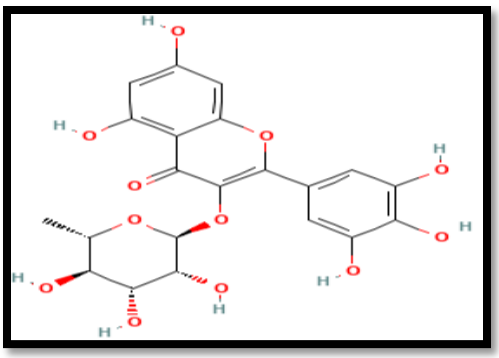
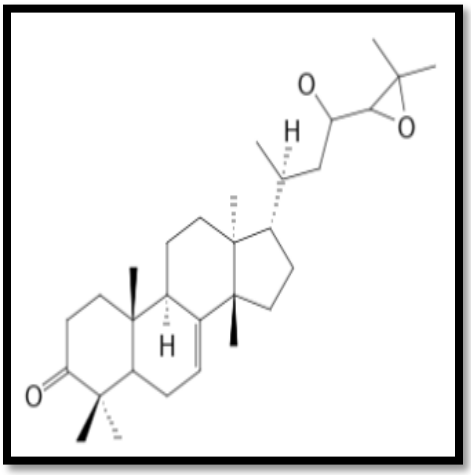


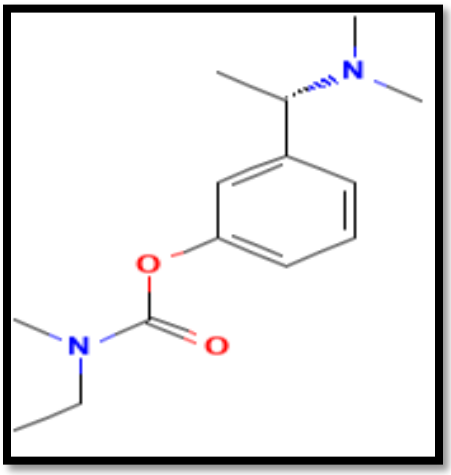
Figure 20 : (a)Methyl gallate



(b) Myricitrin



(c) Nilocitin



(d) Rivastigmine

6.3 Procedure

6.3.1 Drug likeness (or) lipinski rule of five

The Lipinski's rule of five was published in 1997 by Christopher A. Lipinski and is also known as the Pfizer's rule of five or Rule of five (Ro5).Drug-likeness assesses qualitatively the chance for a molecule to become an oral drug with respect to bioavailability. Drug-likeness was established from structural or physiochemical inspections of development compounds advanced enough to be considered oral drug-candidates. It is a rule of thumb to evaluate the drug-likeness and to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. Ro5 depends on four simple physiochemical parameter ranges: the molecular weight (MW), which should be less than

500 Dalton, lipophilicity (LogP)lessthan 5,and number of hydrogen bond donors and acceptors less than 5 and10,respectively, as seen for 90% of orally functional drugs that have obtained phase II clinical status. These parameters are connected with intestinal permeability and aqueous solubility and determine the first step of oral bioavailability. These rules explain molecular properties valuable for a drug's pharmacokinetics in the human body, including their absorption

distribution, metabolism, and excretion (ADME). If a ligand fails to fulfill the parameters of Ro5, then it is highly probable that it will cause trouble if ingested.The violation of 2 or more of these conditions predicts a molecule as a non-orally available drug.

6.3.2 Admet Prediction of Ligands

ADMET analysis of ligands (phytoconstituents) are predicted with the help of pkCSM software (http://biosig.unimelb.edu.au/pkcsm/)from this online platform, absorption, distribution, metabolism, excretion, toxicity profile of the drug candidates can be calculated.

Admet Prediction Through Pkcsm

1. Open the suitable or convenient web browser and type the URL (http://biosig.unimelb.edu.au/pkcsm/) and enter into the home page.
2. Then click the pkCSM option
3. Copy the SMILES string from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and paste in the SMILES box and click the ADMET prediction mode.
4. Results are generated within a minutes and copy the results in word file and save.

6.3.3 Interpretation of Admet Results Via Pkcsm Chart

Absorption

1. Water Solubility

The water solubility of a compound (logS) reflects the solubility of the molecule in water at 25°C. Lipid-soluble drugs are less well absorbed than water-soluble ones, especially when they are enteral. This model is built using experimental water solubility measurements of 1708 molecules

Interpretation of results:

The predicted water solubility of a compound is given as the logarithm of the molar concentration (log mol/L).

2. Caco-2 Permeability

The Caco-2 cell line is composed of human epithelial colorectal adenocarcinoma cells. The Caco-2 monolayer of cells is widely used as an in vitro model of the human intestinal mucosa to predict the absorption of orally administered drugs. This model is based on 674 drug like molecules with Caco-2 permeability values and predicts the logarithm of the apparent permeability coefficient (log Papp; log cm/s).

Interpretion of results:

A compound is considered to have a high Caco-2 permeability if it has a Papp > 8 x 10^{-6} cm/s. For the pkCSM predictive model, high Caco-2 permeability would translate in predicted values > 0.90.

3. Intestinal Absorption (Human)

The Intestine is normally the primary site for absorption of a drug from an orally administered solution. This method is built to predict the proportion of compounds that were absorbed through the human small intestine.

Interpret of results:

For a given compound it predicts the percentage that will be absorbed through the human intestine. A molecule with an absorbance of less than 30% is considered to be poorly absorbed.

4. Skin Permeability

Skin permeability is a significant consideration for many consumer products efficacy, and of interest for the development of transdermal drug delivery. This predictor was built using 211 compounds whose in vitro human skin permeability has been measured.

Interpretation of results:

It predicts whether if given compound is likely to be skin permeable, expressed as the skin permeability constant logKp (cm/h). A compound is considered to have a relatively low skin permeability if it has a logKp > -2.5.

5. P-glycoprotein substrate

The P-glycoprotein is an ATP-binding cassette (ABC) transporter. It functions as a biological barrier by extruding toxins and xenobiotics out of cells. P-glycoprotein transport screening is performed using transgenic MDR knockout mice and in vitro cell systems. This model was built using 332 compounds that have been characterized for their ability to be transported by Pgp.

Interpretation of results:

The model predicts whether a given compound is likely to be a substrate of Pgp or not.

6. P-glycoprotein I and II inhibitors

Modulation of P-glycoprotein mediated transport has significant pharmacokinetic implications for Pgp substrates, which may either be exploited for specific therapeutic advantages or result in contraindications. This predictive models were build using 1273 and 1275 compounds that have been characterized for their ability to inhibit P-glycoprotein I and P-glycoprotein II transport, respectively.

Interpretation of results:

The predictor will determine is a given compound is likely to be a P-glycoprotein I/II inhibitor.

Distribution

1. VDss (Human)

The steady state volume of distribution (VDss) is the theoretical volume that the total dose of a drug would need to be uniformly distributed to give the same concentration as in blood plasma.

The higher the VD is, the more of a drug is distributed in tissue rather than plasma. It can be affected by renal failure and dehydration. This predictive model was built using the calculated steady state volume of distribution (VDss) in humans from 670 drugs. The predicted logarithm of VDss of a given compound is given as the log L/kg.

Interpretation of results:

VDss is considered low if below 0.71 L/kg (log VDss < -0.15) and high if above 2.81 L/kg (log VDss > 0.45).

2. Fraction Unbound (Human)

Most drugs in plasma will exist in equilibrium between either an unbound state or bound to serum proteins. Efficacy of a given drug may be affect by the degree to which it binds proteins within blood, as the more that is bound the less efficiently it can traverse cellular membranes or diffuse. This predictive model was built using the measured free proportion of 552 compounds in human blood (Fu).

Interpretation of results:

For a given compound the predicted fraction that would be unbound in plasma will be calculated.

3. BBB permeability

The brain is protected from exogenous compounds by the blood-brain barrier (BBB). The ability of a drug to cross into the brain is an important parameter to consider to help reduce side effects and toxicities or to improve the efficacy of drugs whose pharmacological activity is within the brain. Blood-brain permeability is measured in vivo in animals models as logBB, the logarithmic ratio of brain to plasma drug concentrations. This predictive model was built using 320 compounds whose logBB has been experimentally measured.

Interpretation of results:

For a given compound, a logBB > 0.3 considered to readily cross the blood-brain barrier while molecules with logBB < −1 are poorly distributed to the brain.

4. CNS permeability

Measuring blood brain permeability can difficult with confounding factors. The blood-brain permeability-surface area product (logPS) is a more direct measurement. It is obtained from in

situ brain perfusions with the compound directly injected into the carotid artery. This lacks the systemic distribution effects which may distort brain penetration. This predictive model was built using 153 compounds whose logPS has been experimentally measured.

Interpretation of results:

Compounds with a logPS > -2 are considered to penetrate the Central Nervous System (CNS), while those with logPS < -3 are considered as unable to penetrate the CNS.

Metabolism

1. CYP2D6/CYP3A4 substrate

The cytochrome P450's are responsible for metabolism of many drugs. However inhibitors of the P450's can dramatically alter the pharmacokinetics of these drugs. It is therefore important to assess whether a given compound is likely to be a cytochrome P450 substrate. The two main isoforms responsible for drug metabolism are 2D6 and 3A4. These models were built using 671 compounds whose metabolism by each cytochrome P450 isoform has been measured.

Interpretation of results:

The predictor will assess whether a given molecule is likely to be metabolized by either P450.

2. Cytochrome P450 inhibitors

Cytochrome P450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by the cytochrome P450's, and some can be activated by it. Inhibitors of this enzyme, such as grapefruit juice, can affect drug metabolism and are contraindicated. It is therefore important to assess a compounds ability to inhibit the cytochrome P450. Models for different isoforms were built (CYP1A2/CYP2C19/CYP2C9/CYP2D6/CYP3A4) using from over 14000 to 18000 compounds whose ability to inhibit the cytochrome P450 has been determined. A compound is considered to be a cytochrome P450 inhibitor if the concentration required to lead to 50% inhibition is less than 10 uM.

Interpretation of results:

The predictors will assess a given molecule to determine whether it is likely going to be a cytochrome P450 inhibitor, for a given isoform.

Excretion

1. Total Clearance

Drug clearance is measured by the proportionality constant CLtot, and occurs primarily as a combination of hepatic clearance (metabolism in the liver and biliary clearance) and renal clearance (excretion via the kidneys). It is related to bioavailability, and is important for determining dosing rates to achieve steady-state concentrations. This predictor was built using the total clearance data for 398 compounds.

Interpretation of results:

The predicted total clearance log(CLtot) of a given compound is given in log(ml/min/kg).

2. Renal OCT2 substrate

Organic Cation Transporter 2 is a renal uptake transporter that plays an important role in disposition and renal clearance of drugs and endogenous compounds. OCT2 substrates also have the potential for adverse interactions with co-administered OCT2 inhibitors. Assessing a candidates potential to be transported by OCT2 provides useful information regarding not only its clearance but potential contraindications. This model was built using 906 compounds whose transport by OCT2 has been experimentally measured.

Interpretation of results:

The predictor will assess whether a given molecule is likely to be an OCT2 substrate.

Toxicity

1. AMES toxicity

The AMES test is a widely employed method to assess a compounds mutagenic potential using bacteria. A positive test indicates that the compound is mutagenic and therefore may act as a carcinogen. This predictive model was built on the results of over 8000 compounds Ames tests.

Interpretation of results:

It predicts whether a given compound is likely to be Ames positive and hence mutagenic.

2. Maximum Tolerated Dose

The maximum recommended tolerated dose (MRTD) provides an estimate of the toxic dose threshold of chemicals in humans. The model is trained using 1222 experimental data points

from human clinical trials and predicts the logarithm of the MRTD (log mg/kg/day). This will help guide the maximum recommended starting dose for pharmaceuticals in phase I clinical trials, which are currently based on extrapolations from animal data.

Interpretation of results

For a given compound, a MRTD of less than or equal to 0.477 log(mg/kg/day) is considered low, and high if greater than 0.477 log(mg/kg/day).

3. hERG I and II Inhibitors

Inhibition of the potassium channels encoded by hERG (human ether-a-go-go gene) are the principal causes for the development of acquire long QT syndrome - leading to fatal ventricular arrhythmia. Inhibition of hERG channels has resulted in the withdrawal of many substances from the pharmaceutical market. These predictors were built using hERG I and II inhibition information for 368 and 806 compounds, respectively.

Interpretation of results

The predictor will determine if a given compound is likely to be a hERG I/II inhibitor.

4. Rat LD50

It is important to consider the toxic potency of a potential compound. The lethal dosage values (LD50) are a standard measurement of acute toxicity used to assess the relative toxicity of different molecules. The LD50 is the amount of a compound given all at once that causes the death of 50% of a group of test animals.

Interpretation of results:

The model was built on over 10000 compounds tested in rats and predicts the LD50 (in mol/kg).

5. Oral Rat Chronic Toxicity

Exposure to low-moderate doses of chemicals over long periods of time is of significant concern in many treatment strategies. Chronic studies aim to identify the lowest dose of a compound that results in an observed adverse effect (LOAEL), and the highest dose at which no adverse effects are observed (NOAEL). This predictor was built using the LOAEL results from 567mpounds.

Interpretation of result

For a given compound, the predicted log Lowest Observed Adverse Effect (LOAEL) in log(mg/kg bw/day) will be generated. The LOAEL results need to be interpreted relative to the bioactive concentration and treatment lengths required.

6. Hepatotoxicity

Drug-induced liver injury is a major safety concern for drug development and a significant cause of drug attrition. This predictor was built using the liver associated side effects of 531 compounds observed in humans. A compound was classed as hepatotoxic if it had at least one pathological or physiological liver event which is strongly associated with disrupted normal function of the liver.

Interpretation of result

It predicts whether a given compound is likely to be associated with disrupted normal function of the liver.

7. Skin Sensitisation

Skin sensitisation is a potential adverse effect for dermally applied products. The evaluation of whether a compound, that may encountered the skin, can induce allergic contact dermatitis is an important safety concern. This predictor was built using 254 compounds which have been evaluated for their ability to induce skin sensitisation.

Interpretation of result

It predicts whether a given compound is likely to be associated with skin sensitisation.

8. T. Pyriformis toxicity

T. Pyriformis is a protozoa bacteria, with its toxicity often used as a toxic endpoint. This method was build using the concentration of 1571 compounds required to inhibit 50% of growth (IGC50).

Interpretation of result

For a given compound, the pIGC50 (negative logarithm of the concentration required to inhibit 50% growth in log ug/L) is predicted, with a value >-0.5 log ug/L is considered toxic.

9. Minnow toxicity

The lethal concentration values (LC50) represent the concentration of a molecule necessary to cause the death of 50% of the Flathead Minnows. This predictive model was built on LC50 measurements for 554 compounds.

Interpretation of result

For a given compound, a log LC50 will be predicted. LC50 values below 0.5 mM (log LC50 < -0.3) are regarded as high acute toxicity.

6.4 Docking Procedure

Step 1:

- Spdb viewer**(Target protein preparation)**
- Open -(spdbv Application) Click File - Open pdb file
- Select unwanted amino and residues
- Go to Build option - Remove selected residues
- Go to the file –Save - Current layer
- Save the protein as (.pdb) format

Step: 2

- BIOVIA Discovery Studio Visualizer **(Ligands collection & Clustering)**
- File - New- Molecules window
- File - Insert from - Files
- Select all ligands
- File - Save
- Saveligand cluster as (.sdf) format
- Save as type (MDL Mol/ SD Files)

Step: 3

- PyRx Software **(Docking Process)**
- Edit – Preferences…
- In " Work space section – Browse – Click work space folder
- 3 Folders will be created in work space Folder automatically
- Close the PyRx application and again open it
- Click - File- Import - Chemical Table files - SDF
- Click on NEXT
- Select "Ligand Cluster" in "Work space" folder
- All the ligands cluster will be imported
- Then Right click on any one of the ligand and select "Minimize all"
- Again, Right click on any one of the Ligand and select "Convert all to Auto dock ligand (pdbqt)"
- In "Auto dock" section all the ligands will be converted to (.pdbqt) format.
- **Now macromolecules need to be imported**
- File - Load molecules
- Go to (working space) -Then Select the Processed Protein - Open
- Now the Macro molecules needs to be converted to .pdbqt format
- In "molecule" Section Right click the targeted protein
- Auto dock - Make macromolecule
- In the "Auto dock" section the target will be converted to (.Pdbqt) format
- Select "Vina Wizard"– "Select molecules" section
- Then select all the Ligands and Macromolecules in (.pdbqt) format
- Then click "forward"
- Then click "Maximize" to Cover Proteins by grid box
- Then click "Forward"
- The docking process will be Started
- Click on the "Save as comma-separated values (csv)"
- Save the file name as Trial I results .csv
- Hint: Same the file extension in (.csv) format

Step 4:

- PyMOL Application **(Building the Protein ligand complex)**
- File - Open - Working space (Trial 1 Folder) - Protein target
- Trial 1 - Macromolecules – Protein target.pdbqt (or)
- Simply drag the .pdbqt file to PyMOL Window
- Then click "File" - Export molecule - Multi-File - One single file - Save
- File (or) Save name as (protein-ligand complex .pdb)
- Save as type: PDB (*.pdb) files
- Hint: Save the file with extension in (.pdb) format

Step: 5

- BIOVIA discovery studio visualizer **(To Study Protein-Ligand interactions)**
- File – Open – Protein ligand complex
- Tools – Receptor ligand interaction – Define & Edit binding sites – From receptor cavities
- Tools – Receptor ligand interaction – View interaction – Show 2D diagram
- Chart – Ramachandran plot, Hydrophobicity plot, Contact plot – H-bond plot
- File – Save as – Image file

6.5 Materials used for *In-vitro* and *In-vivo* studies

6.5.1 Plant Material

- The dried leaves of *Eugenia jambolana*

6.5.2 Calculation of Percentage Yield

The percentage yield was calculated for extracts and major compounds with reference to crude material taken using the formula given below [89]

$$\textbf{Percentage yield} = \frac{\textbf{weight in gram of extract obtained}}{\textbf{weight in gram of plant material}} x\ 100$$

6.5.3 Chemicals & Reagents

s.no	Name of the chemical	Manufacturer
1.	Ethanol-99.9%	Loba Chemie Pvt.Ltd.
2.	Petroleum ether	Himedia Pvt Ltd.
3.	DPPH	Bio pharma Ltd.
4.	Ascorbic acid	Merck KGaA
4.	Mayer's reagent	Spectrum
5.	Dragandroff's reagent	ACS chemicals

6.	Wagner's reagent	Quali tech
7.	Molisch's reagent	ACS chemicals
8.	Benedict's reagent	Fisher scientific
9.	Ferric Chloride	Belfin chemicals
10.	Keller's reagent	Ricca chemicals
11.	Sodium hydroxide	Reagent chemicals
12	Aluminium chloride	Sigma Aldrich
13	Rivastigmine	Medical shop
14	Acetylthiocholine	Calgon Scientific Co, Edappally, Kochi
15	DTNB 5,5'-Dithiobis-(2-nitrobenzoic acid) or Ellman's reagent	Calgon Scientific Co, Edappally, Kochi

Table 7 : List of Chemicals

6.5.4 Instruments

S.no	Name of the instrument	Manufacture (model)
1.	Soxhlet apparatus	United scientific supplies Ltd
2.	HPTLC	Shimadzu
3.	Rotary evaporator	Super fit rotavac R/185
4.	Colorimetry	Vision
5.	UV-VIS Spectrophotometer	Varian Carry 5000
6.	Morris Water Maze	College laboratory

Table 8: List of instruments.

6.5.5 Experimental Animals

The animals were purchased from Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala and were transported to St. Joseph's College of Pharmacy, Cherthala in an air conditioned vehicle in rat cages and were kept in animal house for further experimental purposes. Adult male and female Wistar rats with body weights of 150-250gm were used for the study. The animals were maintained under standard environmental conditions (23-250C, 12 hour light/12 hour dark cycle) and had free access to standard rodent pellet and water ad libitum. The animals were acclimatized in laboratory condition for a week before commencement of the study

6.5.6 Plant Collection And Authentication

The fresh leaves of *Eugenia Jambolana* were collected during the month of December from college premises, Othakalmandapam, Coimbatore, Tamilnadu, India. The plant specimen was authenticated by Dr.M.U. Sharief, scientist 'E'& Head of Office, Botanical survey of India, southern regional centre, Coimbatore.The herbarium specimen with voucher were deposited in department of pharmacognosy for future reference (No.: BSI/SRC/5/23/2021/Tech/25).

6.6 Methods

6.6.1 Preparation and Extraction of Plant Material

The collected leaves of *Eujenia Jambolan* were washed thoroughly with water to remove the dust particles, shade dried and powdered coarsely with a mechanical grinder and stored in an air tight container.

The powder was deffated with Petroleum Ether The powder was extracted by continuous hot Soxhlet extraction using Ethanol 99.99%. After each extraction, the extracts were filtered through whatman filter paper to remove any impurities if present and dried by rotavacum evaporator under controlled temperature and pressure and stored in refrigerator at 4°C for future use.[95,96]

Figure 21: Soxhlet Extraction

6.6.2 Preliminary Phytochemical Analysis

Phytochemicals are the chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not establish as essential nutrients[90,91,92] .

Need of phytochemical analysis is to find the bioactive constituents present in plants having therapeutic importance.

Test for Alkaloids

Small fractions of solvent free extracts were separately stirred with milliliters of dilute hydrochloric acid and filtered; the filtrate is tested with following reagents for the presence of alkaloids.

Wagner's Test (Iodine in potassium iodide solution)

A fraction of the extracts was treated with Wagner's reagent and observed for the formation of a reddish brown precipitate.

Test for Carbohydrate

Extracts were dissolved separately and were tested with Molisch reagent, Fehling's reagent, Benedict's solution for detection of carbohydrates.

Molisch Test (α-naphthol in ethanol)

To 2ml of the extracts, 1ml of α-naphthol solution was added, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet colour ring at the junction of the two liquids revealed the presence of carbohydrates.

Fehling's Test (Fehling's A - Copper sulphate solution, Fehling's B - Sodium potassium tartarate in sodium hydroxide)

A little fraction of filtrate treated with Fehling's solution A and B and then heated on a water bath. Brick red precipitate reveals the presence of reducing sugars.

Test For Glycosides

Glycosides were confirmed by subjecting the acid hydrolysed extract to Legal's test, Borntrager test and Libermann-Burchard's test.

Legal Test

The hydrolysate was dissolved in pyridine and sodium nitro-prusside solution and was added with sodium hydroxide to make it alkaline. The colour change showed the presence of glycosides

Test for Terpenoids

Chloroform Test

A few drops of chloroform and conc. sulfuric acid was added carefully along the sides of test tube to 5ml of the extract resulting to formation of layer and a reddish brown colour shows the presence of terpenoids

Test For Flavonoids

Shinoda's Test

Small quantity of the extract was dissolved in alcohol, to that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

Test For Phenolic Compounds And Tannins

All the dry extracts were dissolved in minimum amount of water, filtered and subject to Ferric chloride test and Gelatin test.

Ferric Chloride Test

Filtrate was added with few drops of ferric chloride. Formation of violet colour precipitate shows the presence of tannins.

Test For Protein And Aminoacid

Biuret Test (Alkaline copper sulphate solution)

2ml of extract was added with 2ml of biuret reagent. Formation of a violet colour showed the presence of proteins.

Ninhydrin Test (Ninhydrin in acetone) 2ml of extract was added with 2ml of ninhydrin reagent. Formation of a blue colour showed the presence of proteins

Test For Saponins

Foam Test

The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15minutes. Formation of 1cm layer of foam shows the presence of saponins.

6.6.3 Estimation of Total Phenolic Content

The total phenolic content was determined spectrophotometrically by Folin-Ciocalteu methods and expressed as mg of Gallic acid equivalent (GAE) per g extract[98] .An aliquot(1 ml) of the ethanolic extract was mixed with 1ml of Folin-Cicalteu reagent. After 5 min, the mixture was neutralized with 10 ml of 7 % aqueous Na_2CO_3 solution followed by the addition of 13ml deionoised water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23^0 C, After 40 which the absorbance of the mixture was measured at 760 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid and expressed as mg of Gallic acid equivalent (GAE) per g extract.[97,99]

6.6.4 HPTLC Screening For Antioxidants

The antioxidant activity of *Eugenia jambolana* plant ethanol extract was done using Hptlc . TLC was performed on 10 cm × 20 cm glass Si60 HPTLCF254 plates in which the mobile phase used was chloroform : methanol : water 70 : 30 : 4 v/v/v for the ethanol extracts .After spotting using micro pipette ,the plate was developed for a distance of 90 mm. The plate was dried at room temperature for 20 min. After this time, the plate was sprayed using 0.5% DPPH solution for 5 sec. Active compounds appeared as yellow-white spots against a purple background. White spots

were visualized under day light and uv under 233nm[100]

6.6.5 IAEC Clearance

The total number of animals used for invivo pharmacological activity, mode of transport, method of study treatment and handling of animals were presented to IAEC and the committee approved the proposal number:**KFMSR/M.Pharm/03/2021** for the proceeding experiments of rats.

6.6.6 Acute Oral Toxicities Studies And Dose Selection

Acute oral toxicity test Acute oral toxicity test was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals, 420. When a single dose of aqueous extract of VT and methanolic extract of VT was orally administered up to a concentration of 2000mg/kg, no adverse effects and mortality were observed. So from the acute oral toxicity test, it can be understood that *Eujenia Jambolana* has low toxicity profile[93,94]

Dose Selected

So the dose selected from the acute toxicity test for the study where, Low Dose: 200mg/kg

High Dose: 400mg/kg

6.6.7 Pharmacological Screening

Evaluation of neuroprotective activity of *Eugenia jambolana* leaves extract on Aluminium tri chloride induced Alzheimer's disease in SD rats

Procedure

Experiments were carried out on male and female SD rats (150-250g). The total 30 rats were divided into 5 groups containing 6 animals in each. All rats were trained in Morris water maze test . For the 4 groups other than Normal control the rat model mimicking AD is produced by administering $AlCl_3$ orally at a dose of 17 mg/kg body weight daily for 4 successive weeksAD-induced rats treated daily orally with *Eugenia jambolana* extract for 21 consecutive days at 2 selected doses ,AD-induced rats treated orally with Rivastigmine (RIVA) (0.3 mg/kg body weight/Day) as reference drug, daily for 21 consecutive days (after stopping $AlCl_3$ administration). The morris water maze test will be performed before induction of disease , after induction of disease , and after treatment with extract and standard drug. The mean of all s

groups are taken with the standard error [98,101]

6.6.8 Groups And Schedules

S. No	GROUPING	TREATMENT	DOSE	SITE
1	Group 1 = normal control	Normal saline	1ml/kg	Oral
2	Group 2 = negative control	$AlCl_3$	17mg/kg	Oral
3	Group 3 = positive control	$AlCl_3$+ Rivastigmine (Standard drug)	17mg/kg +0.3mg/kg	Oral
4	Group 4 = test 1	$AlCl_3$ + EJ extract	17mg/kg+200mg/kg	Oral
5	Group 5= test 2	$AlCl_3$+ EJP extract	17mg/kg+400mg/kg	Oral

Table 9 : Grouping And Schedules

6.6.9 Morris Water maze test

The water maze test is also a widely accepted method for memory test. A circular water pool was filled with milky water kept at 22–25 ºC. An escape platform was submerged below the surface of the water in position. On training trials, the rats were placed in a pool of water and allowed to remain on the platform for 10 s and were then returned to the home cage during the second-trial interval. The rats that did not find the platform within 60 s were placed on the platform for 10 s at the end of trial. Animals were given 4 trials daily for 4 consecutive days. On the 15th day, rats were individually subjected to a probe trial session by removing the platform and were allowed to swim for 120 s to search for the platform.[102,103]

6.6.10 Estimation of Acetylcholinesterase (AChE) Ellman's method

Acetylcholinesterase (AChE) is an enzyme participating in cholinergic neurotransmission. It breaks down acetylcholine which terminates the neurotransmission process. The most common

assay is based on Ellman's method using an alternative substrate acetylthiocholine and 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). The reaction results in production of 5-thio-2-nitrobenzoate that has yellow color due to the shift of electrons to the sulfur atom. The animals The animals were sacrificed, whole brains was removed quickly and placed in ice-cold saline. Then blood centrifuged for 10min at 2000 rpm. and homogenized in 0.1M Phosphate buffer (pH 8). 4ml aliquot of the homogenate is added to a cuvette containing 2.6ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly and absorbance was measured at 412nm in a spectrophotometer. When absorbance reaches a stable value, it was recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance is recorded. Change in the absorbance per minute was determined. Protein estimation was done using folin's method. AChE activity was calculated using the following formula:[104,105,106]

Calculations

The enzyme activity is calculated using the following formula

$$\text{Acetylcholinesterase activity (M/ml) } R = \frac{\boldsymbol{\delta O.D \times \text{Volume of Assay } (5ml)}}{\boldsymbol{E \times \text{mg of protein}}}$$

Where, rate of enzyme activity in n' mole of acetylcholine iodide hydrolysed / min/ mg E Extinction coefficient13600 M-1cm-1

δO.D = change in absorbance

The final reading of enzyme activity is expressed as µ moles/minute/mg tissue**.**

6.7 Statistics

All the values were expressed as mean ±SD. EC50 value was estimated by applying Non-Linear Regression followed by EC50 in Graph Pad Prism . The data was statistically analyzed by one way ANOVA, KRUSWALLIS TEST AND PAIRED T TEST. One way analysis of variance (ANOVA) was used to correlate the statistical difference between the variables. $P< 0.05$, $P<0.001$ was considered to be significant.

7 .RESULTS

7.1 Docking Interaction Analysis

The docking studies were done using PyRx software. The polyphenols derivatives such as Methyl gallate, Myricetrin, Nilocitin, Rivastigmine were docked with selective proteins such as Acetylcholinestrase, Butyrylcholinesterase. The best interaction between ligands and proteins binding affinity values were taken.

S.no	Ligands	Acetylcholinestrase	Butyrylcholinesterase
1	Methyl gallate	-6.8	-6.3
2	Myricetrin	-8.3	-10.2
3	Nilocitin	-8.6	-10.1
4	Rivastigmine	-6.3	-7.1

Table 10 : List of binding energy

7.1.1 Interaction between Acetylcholinestrase and ligands:

Acetylcholinesterase (AChE) is a cholinergic enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves. It immediately breaks down or hydrolyzes acetylcholine (ACh), a naturally occurring neurotransmitter, into acetic acid and choline. The interaction formed between the selectively four ligands and Acetylcholinestrase has been visualized using BIOVIA Discovery studio visualizer tool. The ligand was bonded to protein with 8 van der waal interaction and with conventional hydrogen bond and with 3 alkyl bond and 2 covalent bondThe 2D image ,3D image ,H-Bond plot, were visualized using BIOVIA Discovery studio visualizer tool. Methyl gallate shows similar interaction with the AChE protein with binding energy of(-6.8 kcal mol-¹) than myricitrin (-8.3 kcal mol-¹) and nilocitin (-8.6 kcal mol-¹) when compared to standard Rivastigmine with binding energy (-6.3 kcal mol-¹) shown in Table 10

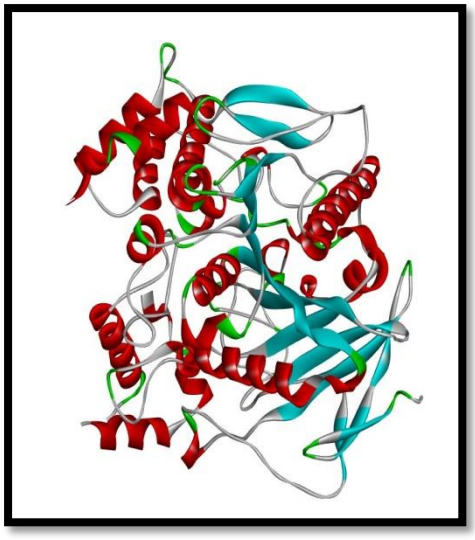


Figure 22 :Structure of Acetylcholinestrase

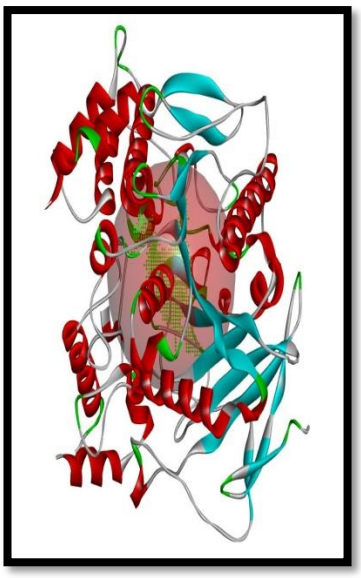


Figure23: Binding site of Ligands with AChE

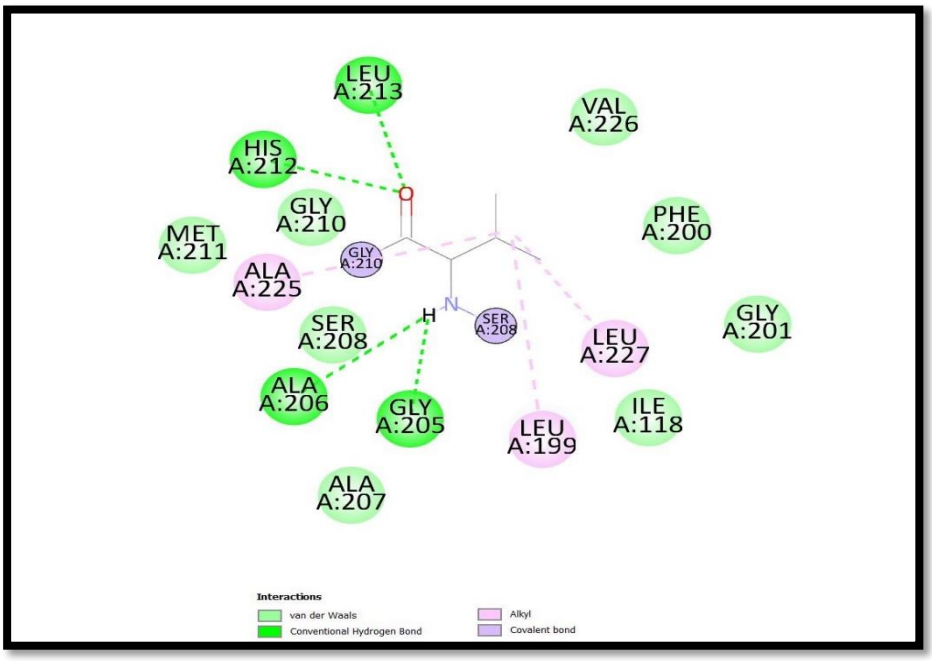


Figure 24 : 2D Complex If Ache And Ligand Interactions

Interaction between Butyrylcholinesteraseand ligands:

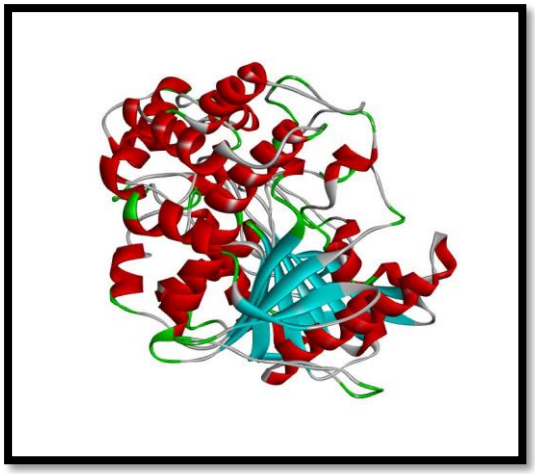


Figure 25 :Structure of Butyrylcholinesterase Ligands

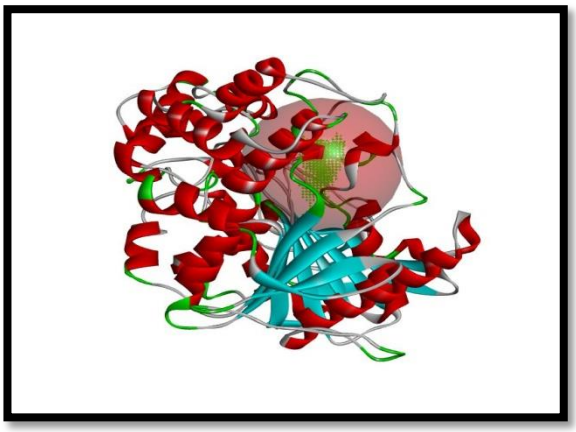


Figure 26:Binding site of BuChE

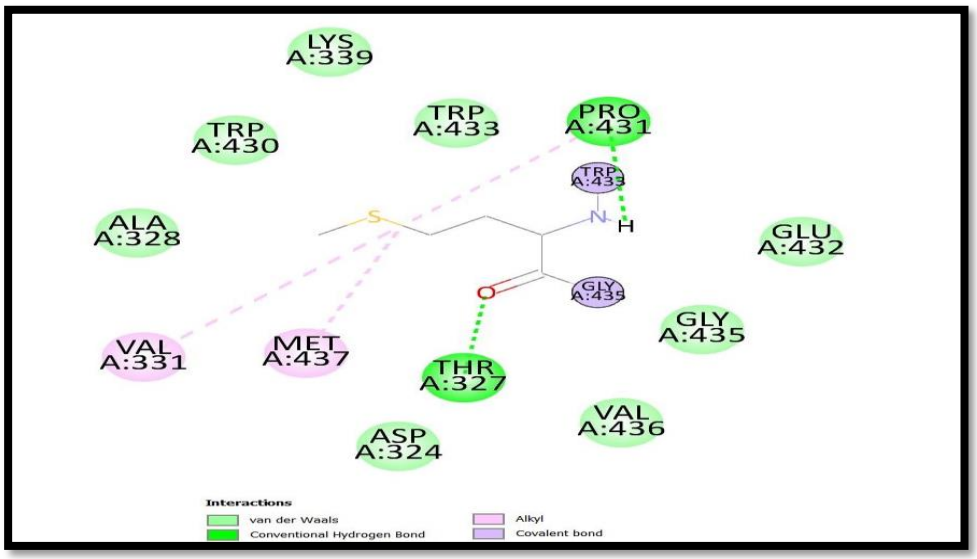


Figure 27 : 2D Complex If Bche And Ligand Interactions

Butyrylcholinesterase also known as BChE, BuChE, pseudocholinesterase, or plasma (cholin)esterase,is a nonspecific cholinesterase enzyme that hydrolyses many different choline-based esters. In humans, it is made in the liver, found mainly in blood

plasma, and encoded by the *BuChE* gene.The interaction formed between the selectively four ligands and Butyrylcholinesterase has been visualized using BIOVIA Discovery studio visualizer tool. The ligand was bonded to protein with 8 van der waal interaction and with conventional hydrogen bond and with 3 alkyl bond and 2 covalent bond The 2D image ,3D image ,H-Bond plot, were visualized using BIOVIA Discovery studio visualizer tool. Methyl gallate shows similar interaction with the BuChE protein with binding energy of(-6.3 kcal mol^{-1}) than Myricitrin (-10.2 kcal mol^{-1}) and Nilocitin (-10.1 kcal mol^{-1}) when compared to standard Rivastigmine with binding energy (-7.1 kcal mol^{-1}) shown in Table 10

7.2 ADMET PREDICTION OF LIGANDS

7.2.1 Predicted absorption profile of ligands

S.no	Ligands	Water solubility(log mol/L)	Caco2 permeability(log Papp in 10^{-6} cm/s)	Intestinal absorption(% Absorbed)	Skin Permeability(log Kp)
1.	Methyl gallate	-2	-0.056	76.635	-2.771
2.	Myricetrin	-2.892	-0.982	43.334	-2.73
3.	Niloticin	-6.419	-1.26	95.753	-3.283
4	Rivastigmine	-2.347	1.569	88.456	-2.882

S.no	Ligands	P-glycoprotein substrate (Yes/No)	P-glycoprotein I inhibitor (Yes/No)	P-glycoprotein II inhibitor (Yes/No)
1.	Methyl gallate	YES	YES	YES
2.	Myricetrin	NO	NO	YES
3.	Niloticin	NO	NO	YES
4	Rivastigmine	NO	NO	NO

Table 11 : Absorption Profile of Ligand

Predicted absorption profile of ligands

1. Water Solubility:

Methyl gallate is more water solubility than other phytoconstituents and standard Rivastigmine.

2. Caco-2 Permeability:

Conclusions of the Caco-2 permeability shows myricetrin and Niloticin passes the reference value of (log Papp in 10^{-6} cm/s) > 0.90.

3. Intestinal Absorption (Human):

Methyl gallate Myricetrin and Nilocitin are absorbed greater than the reference value of 30%. But specifically Niloticin compound are shows 95.7% greater absorption among them. Absorption of standard drug Rivastigmine shows 88.45% of intestinal absorption.

4. Skin Permeability:

All phytoconstituents are considered to have a Skin Permeability from the reference value log Kp> -2.5. Skin Permeability of nilocitin has high value -3.283 when compared to standard drug Rivastigmine which shows -2.882.

5. P-glycoprotein substrate:

Except Methyl gallate compound the other phytoconstituents are not likely to be a Substrate of P-glycoprotein which is an ATP- binding cassette (ABC) transporter.

6. P-glycoprotein I inhibitors:

Methyl gallate inhibits P-glycoprotein-I while other two compounds are can't be inhibit P-glycoprotein-I like standard Rivastigmine

7. P-glycoprotein II inhibitors:

From this prediction more over all the Phytoconstituents inhibits P-glycoprotein- II but standard drug Rivastigmine do not inhibits P-glycoprotein-II

7.2.2 Predicted Distribution profile of ligands

S.no	Ligands	VDss (human) (log L/kg)	Fraction unbound (human) (Fu)	BBB permeability(log BB)	CNS permeability(log PS)
1.	Methyl gallate	0.355	0.615	-1.046	-3.376
2.	Myricetrin	1.552	0.182	-1.046	-3.376
3.	Nilocitin	0.191	0	-0.008	1.34
4	Rivastigmine	0.625	0.538	0.508	-2.255

Table 12: distribution profile of ligands

Predicted Distribution profile of ligands

1. VDss-Volume of Distribution(Human):

Myricetrin is a highly volume of distribution 1.552(log L/kg) in blood plasma lies between the reference value of 0.71 to 2.81 (log VDss> 0.45).The standard drug Rivastigmine has Volume of distribution 0.625 (log L/kg) in blood plasma.

2. Fraction Unbound (Human):

Except Nilocitin compound while other two phytoconstituents shows greater fraction unbound value in plasma. Methylgallate has high fraction unbound compared to standard rivastigmine wth value of 0.615

3. BBB permeability:

More over all the compounds has poor permeability to cross Blood brain barrier (BBB) from the reference value of logBB> 0.3 considered to readily cross the blood-brain barrier while molecules with logBB< −1 are poorly distributed to the brain. But specifically two constituents do not cross BBB i.e, Methyl Gallate and Myricetrin, but Nilocitin has a permeability to cross BBB with a value of -0.008

4. CNS permeability:

More over all the compounds do not posses CNS permeability from the reference value of logPS> -2 are considered to penetrate the Central Nervous System (CNS), while those with logPS< -3 are considered as unable to penetrate the CNS. But specifically Nilocitin has CNS permeability shows -1.34 lies between the reference value .

7.2.3 Predicted Metabolism Profile of Ligands

S.no	Ligands	CYP2D6 substrate (Yes/No)	CYP3A4 substrate (Yes/No)	CYP1A2 inhibitor (Yes/No)	CYP2C19 inhibitor (Yes/No)
1.	Methyl gallate	No	No	No	No
2.	Myricetrin	No	No	No	No
3.	Nilocitin	No	Yes	No	No
4	Rivastigmine	No	No	No	No

S.no	Ligands	CYP2C9 inhibitor (Yes/No)	CYP2D6 inhibitor (Yes/No)	CYP3A4 inhibitor (Yes/No)
1.	Methyl gallate	No	No	No
2.	Myricetrin	No	No	No
3.	Nilocitin	No	No	No
4	Rivastigmine	No	Yes	No

Table 13 :Metabolism Profile of Ligands

1. CYP2D6 substrate:

All the compounds are not likely to be metabolized by the Substrate of CYP2D6, except standard rivastigmine ,which is a isoforms of cytochrome P450 responsible for drug metabolism

2. CYP3A4 substrate:

Expect Nilocitin and the other phytoconstituents are not likely to be metabolized by the substrate of CYP3A4.Standard drug Rivastigmineis also not likely to be metabolized by the SubstrateofCYP3A4.

7.2.4 Predicted Excretion Profile of Ligands

S.no	Ligands	Total Clearance (log ml/min/kg)	Renal OCT2 substrate (Yes/No)
1.	Methyl gallate	0.635	No
2.	Myricetrin	0.303	No
3.	Nilocitin	0.207	No
4	Rivastigmine	0.557	No

Table 14 : Excretion Profile of Ligand

1. Total Clearance:

All the compounds are considered to have a significant total clearance value, whereas one Phytoconstituents i.e, Methyl gallate shows the greater clearance value of 0.635 than standard Rivastigmine 0.557

2. Renal OCT2 substrate:

All the compounds are not likely to be a Substrate of Organic Cation Transporter 2 (OCT2) that plays an important role in disposition and renal clearance of drugs and endogenous compounds.

7.2.5 Predicted Toxicity Profile of Ligands

S.no	Ligands	AMES toxicity(Yes/No)	Max. tolerated dose (human) (log mg/kg/day)	hERG I inhibitor (Yes/No)	hERG II inhibitor (Yes/No)	Oral Rat Acute Toxicity (LD50) (mol/kg)
1.	Methyl gallate	No	-0.296	No	No	1.898
2.	Myricetrin	No	-0.727	No	No	2.139
3.	Nilocitin	No	0.042	No	No	2.537
4	Rivastigmine	No	0.382	No	No	3.402

S.no	Ligands	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/ day)	Hepatoto xicity (Yes/No)	Skin Sensitisation(Yes/No)	*T.Pyriformis* toxicity (log ug/L)	Minnow toxicity (log mM)
1.	Methyl gallate	2.432	No	No	0.195	2.871
2.	Myricetrin	3.383	No	No	0.285	5.997
3.	Nilocitin	1.718	No	No	0.457	-0.62
4	Rivastigmine	1.163	No	No	0.517	1.369

Table 15 : Toxicity Profile of Ligands

1. AMES toxicity:

All compounds are considered not to be a mutagenic and therefore not act as a carcinogen.

2. Maximum Tolerated Dose:

All compounds are analysed for the Maximum Recommended Tolerated Dose (MRTD) which provides an estimate of the toxic dose threshold of chemicals in humans.

3. hERG I Inhibitors:

All the compounds are not likely to inhibit hERG I (human ether-a-go-go gene) which are the principal causes for the development of acquire long QT syndrome - leading to fatal ventricular arrhythmia.

4. hERG II Inhibitors:

More over all the Phytoconstituents are not likely to inhibit hERG II

5. Oral Rat Acute Toxicity (LD50):

All compounds are analysed for the toxic potency of a potential compound. The lethal dosage values (LD50) are a standard measurement of acute toxicity used to assess the relative toxicity of different molecules.

6. Oral Rat Chronic Toxicity (LOAEL):

All compounds are analysed for the lowest dose of a compound that results in an observed adverse effect (LOAEL), and the highest dose at which no adverse effects are observed (NOAEL).

7. Hepatotoxicity:

All Phytoconstituents are considered not to be a Hepatotoxic.

8. Skin Sensitisation:

All compounds are analysed and not likely to be associated with skin sensitisation. Whether it may encounters the skin or may induce allergic contact dermatitis which is an important safety concern.

9. T. Pyriformis toxicity:

All compounds are analysed and not likely to be associated with T. Pyriformis toxicity by using the reference value of > -0.5 log ug/L which is considered to be toxic.

10. Minnow toxicity:

More over all the Phytoconstituents are considered likely to be associated with Minnow toxicity but specifically one phytoconstituents Nilocitin are not likely with minnow toxicity. Other two phytoconstituent and standard rivastigmine are considered to be toxic.

7.3 EVALUATION OF DRUG –LIKENESS OR LIPINSKI RULE OF FIVE

S.no	Ligands	Molecular mass (<=500 dalton)	LogP (<=5)	Hydrogen bond donors (<=5)	Hydrogen bond acceptors (<=10)	Topological Polar Surface Area Å^2 (<=140)	Number of violations
1.	Methyl gallate	184.147	0.59	5	3	73.819	No
2.	Myricetrin	464.379	0.194	8	12	183.901	Yes (3)
3.	Nilocitin	456.711	6.725	1	3	201.673	Yes (2)
4	Rivastigmine	250.342	2.7597	0	3	109.146	No

Table 16 : drug likeness profile of ligands

Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules

1. Molecular mass less than 500 Dalton
2. High lipophilicity (expressed as LogP less than 5)
3. Less than 5 hydrogen bond donors
4. Less than 10 hydrogen bond acceptors
5. Molar refractivity should be between 40-130

Ligand listed in the above must obey the Lipinski rule of 5 so that the chance for a molecule to become an oral drug with respect to bioavailability. The violation of two or more condition predict a molecule as a non-orally available drug .phytoconstituent Methyl gallate shows no violation like standard Rivastigmine and phytoconstituent Myricetrin and Nilocitin has violation 3 and 2 respectively hence they are not reccomented as oral drug.

7.4 Extraction of *Eugenia Jambolana*

The 1000g powdered leaves of *Eugenia jambolana* were subjected to extraction with ethanol. The yield of ethanolic extract was 80 g. The obtained extract was dark green in colour.

7.5 Preliminary Phytochemical Analysis

The preliminary phytochemical analysis methanolic extract reveals that the presence of alkaloid, flavonoid, carbohydrate,phenols, tannins and cardiac glycoside.

S. No	Phytochemicals	Ethanol Extract
1.	Test for Alkaloid	+
2.	Test for flavanoids	+
3.	Test for Saponin	-
4.	Test for Carbohydrate	+
5.	Test for Protein	-
6.	Test for Phenol	+
7.	Test for Sterol	-
8.	Test for Tannin	+
9.	Test for Cardiac Glycoside	+

Table 17: Preliminary Phytochemical Analysis

7.6 Estimation of Total Phenol Content

Total phenol content was determined in comparison with standard Gallic acid and the results were expressed in terms of mg GAE/g dry extract. The phenol content in ethanol extract was found to be 19.9473

Sl No.	Concentration (µg/ml)	Mean absorbance (760 nm)
1.	20	0.1566
2.	40	0.2391
3.	60	0.3123
4.	80	0.4042
5.	100	0.457
6.	*Eugenia jambolana* ethanolic extract (100 µg/ml)	0.1595

Table 18 : Absorbance of standard gallic acid and extract

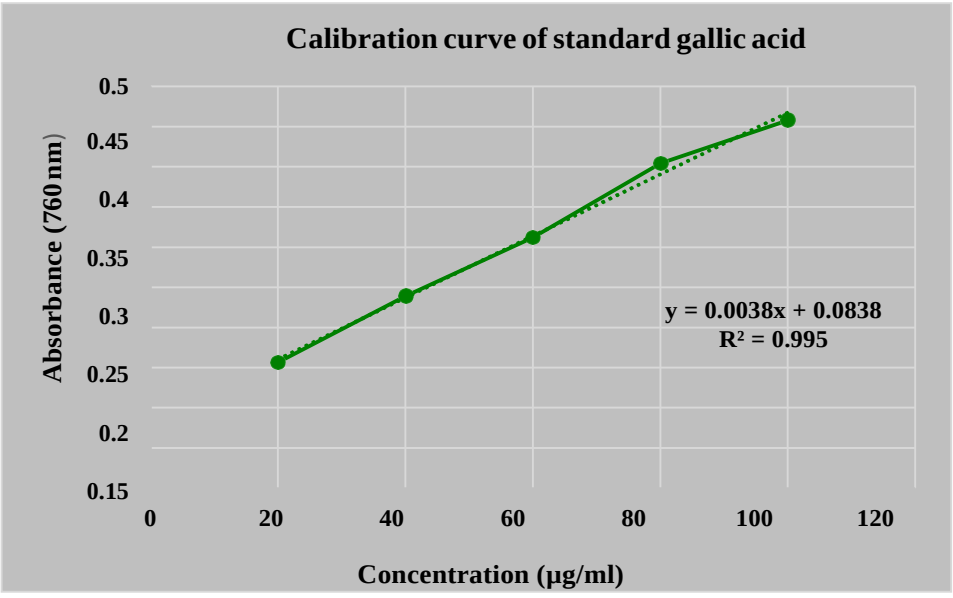


Figure 28: Calibration curve of standard Gallic acid

Total phenolic content of ethanolic extract of *Eujenia jambolana*

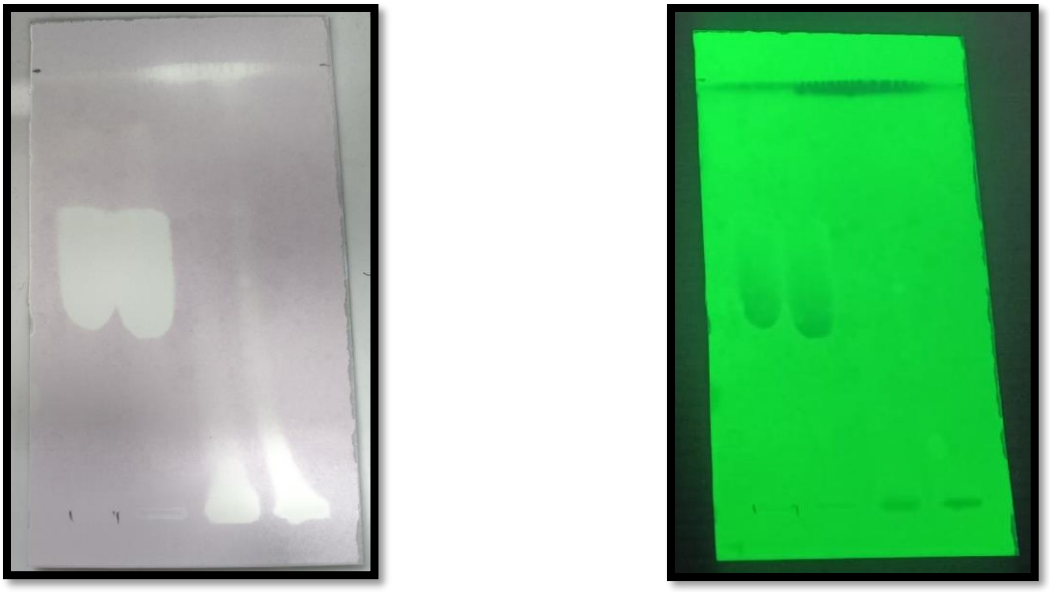
Extract	**Phenolic content- gallic acid equivalent (µg/ml)**
Ethanolic (100 µg/ml)	19.947

Table 19 : Total Phenolic Content

7.7 Antioxidant Activity on HPTLC

Since many of the constituents of herbal extracts possess an antioxidative capacity, it is believed that this property may be involved, at least in part, in the antineurodegenerative mechanism of the herbal extract. The second part of the experiment was focused on the chromatographic identification of DPPH∗ scavengers, based partially on the fingerprinting conditions.

Compounds that exhibit antiradical potential show up as yellow to white spots against a purple background. We observed the plates after 1 min, 30 min, 1 h, 2 h, 5 h, 10 h, and 24 h from the time of immersion of the plate in 0.5% DPPH∗ solution. After 1 min, the ethanol extracts of two showed areas of activity on high Rf at 1.44 and 1.55 respectively for both applied spots. For the standard ascorbic acid showed areas of activity on high Rf at 1.53 and 1.56. The results for all species remain in good agreement with the data referring to the spectrophotometric DPPH∗ assay method with EC_{55} value 50 µg/m



(a) **(b)**

Figure 29 :Photograph of TLC plate after chromatography of standard Ascorbic acid and *Eugenia jambolan* extract (a) white light (b) 254nm

Figure 30 : Graph illustrating percentage area inhibition of standard ascorbic acid and extract

HPTLC – 3D Diagram of EJ Extract And Standard

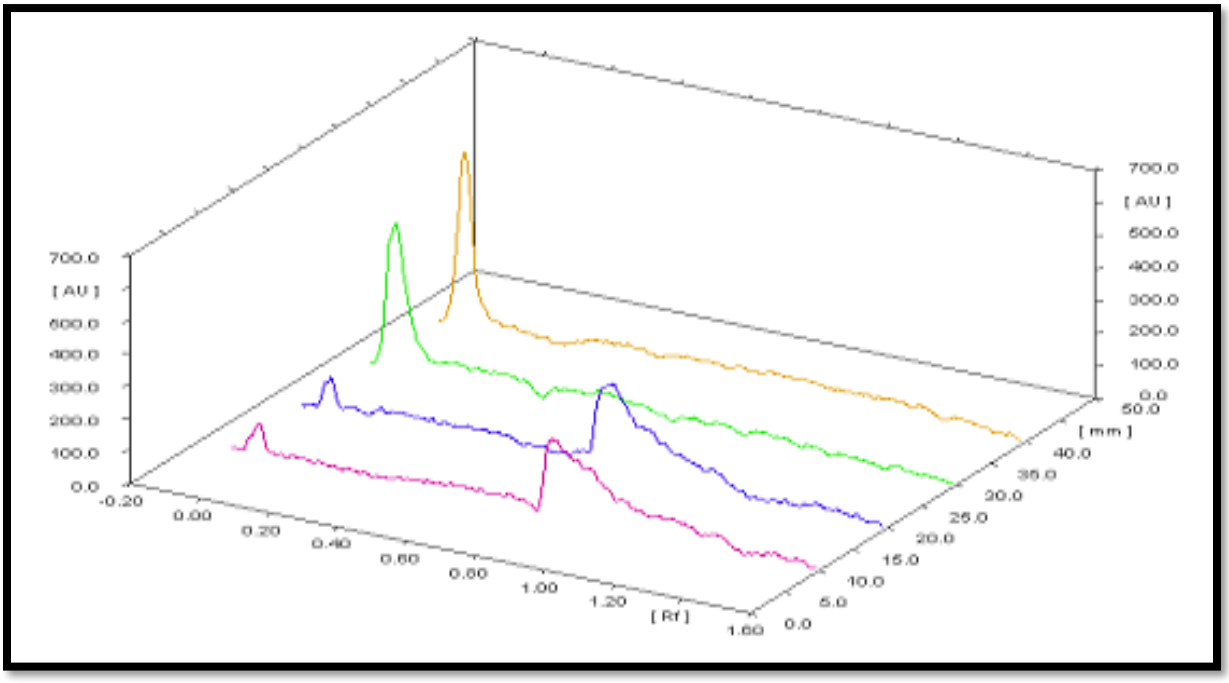


Figure 31 : 3D Diagram of Peaks of EJ Extract And Ascorbic Acid

HPTLC of standard at 254nm

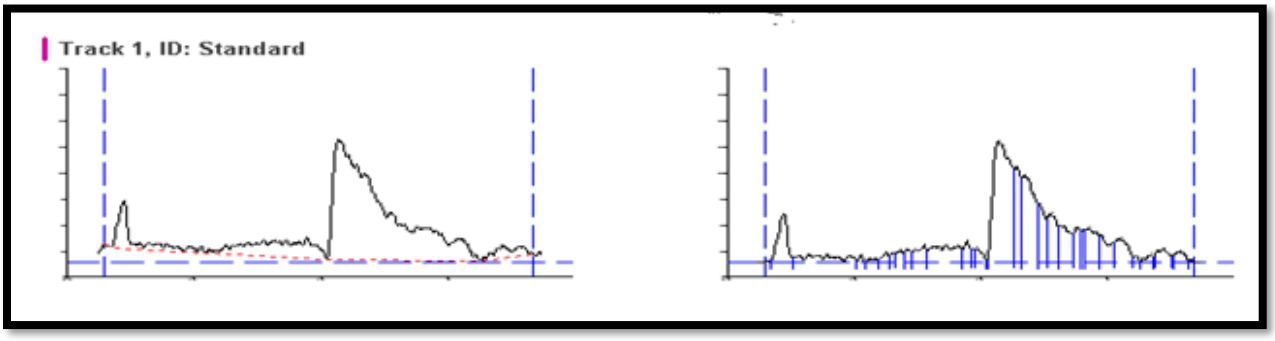


Figure 32 : Peaks of Standard I at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	3.0	-0.02	92.0	9.66	0.01	9.4	1512.4	7.16	unknown *
2	0.26	1.8	0.28	11.6	1.22	0.30	0.1	88.5	0.42	unknown *
3	0.35	4.6	0.38	16.8	1.76	0.39	10.0	194.6	0.92	unknown *
4	0.41	14.7	0.44	20.7	2.17	0.45	16.9	289.6	1.37	unknown *
5	0.48	19.1	0.53	31.0	3.25	0.54	21.8	597.7	2.83	unknown *
6	0.68	24.7	0.69	38.7	4.06	0.72	24.2	492.2	2.33	unknown *
7	0.72	25.0	0.74	25.5	2.68	0.77	0.9	362.2	1.72	unknown *
8	0.78	2.1	0.82	229.6	24.11	0.89	176.4	7628.1	36.13	unknown *
9	0.91	158.5	0.93	165.2	17.34	0.98	111.5	3882.9	18.39	unknown *
10	1.01	81.7	1.03	91.4	9.59	1.05	66.9	1597.3	7.57	unknown *
11	1.11	53.8	1.13	65.8	6.90	1.15	59.8	906.2	4.29	unknown *
12	1.16	61.0	1.18	63.1	6.62	1.22	49.8	1397.5	6.62	unknown *
13	1.27	35.9	1.30	43.9	4.61	1.36	8.0	1091.5	5.17	unknown *
14	1.38	0.4	1.42	12.8	1.34	1.43	8.9	170.1	0.81	unknown *
15	1.44	9.7	1.47	24.8	2.60	1.51	10.2	534.3	2.53	unknown *
16	1.51	10.8	1.53	19.6	2.06	1.57	2.0	365.7	1.73	unknown *

Table 20 : Represented the Standard Ascorbic Acid in track 1. Total sixteen peaks were found with Rf values ranging from -0.07 to 1.51

HPTLC of standard at 254nm

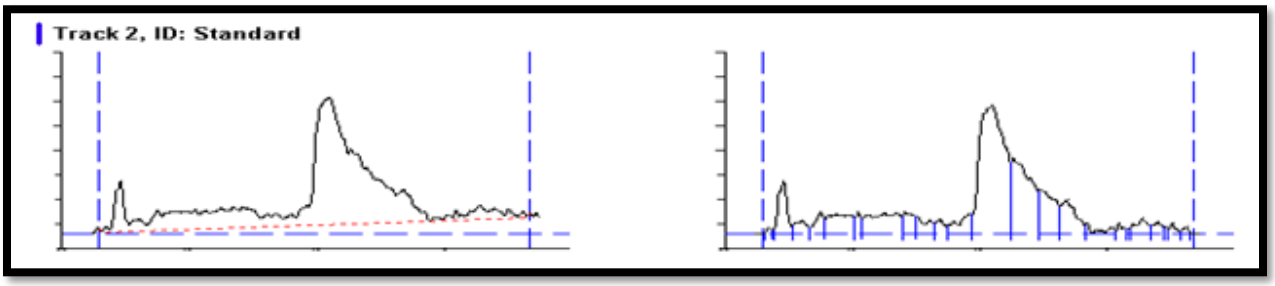


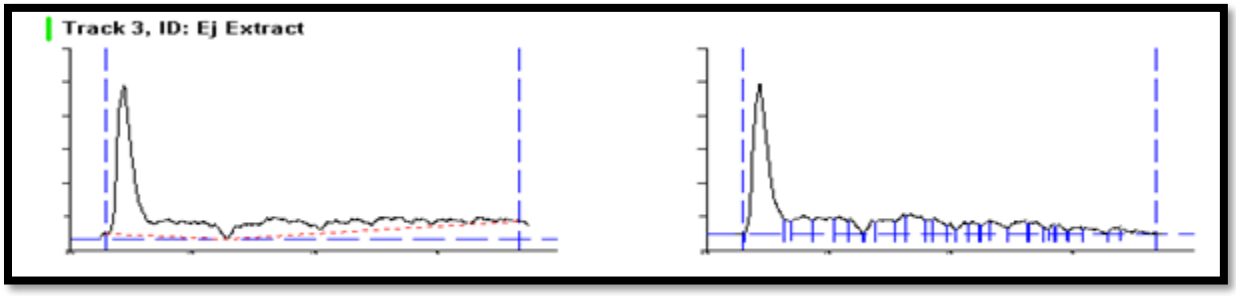
Figure 33 :Peaks of standard II at 254nm

Rf values of standard at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.09	0.0	-0.08	11.0	1.26	-0.06	5.1	100.2	0.39	unknown *
2	-0.06	6.3	-0.02	104.1	11.91	0.01	14.2	1655.9	6.45	unknown *
3	0.02	14.7	0.05	24.9	2.85	0.09	12.4	554.8	2.16	unknown *
4	0.09	12.8	0.13	40.9	4.68	0.14	29.4	678.3	2.64	unknown *
5	0.26	33.4	0.28	40.8	4.67	0.29	30.3	459.7	1.79	unknown *
6	0.45	34.7	0.46	42.2	4.83	0.51	33.3	912.7	3.56	unknown *
7	0.57	20.7	0.60	26.4	3.02	0.62	15.0	463.4	1.81	unknown *
8	0.72	38.3	0.80	261.1	29.87	0.88	144.2	12006.1	46.79	unknown *
9	0.88	145.1	0.89	152.6	17.46	0.98	88.2	5325.8	20.76	unknown *
10	1.06	55.3	1.09	67.1	7.68	1.17	17.6	1900.4	7.41	unknown *
11	1.28	5.6	1.30	20.6	2.36	1.33	8.2	220.0	0.86	unknown *
12	1.34	8.3	1.39	28.2	3.23	1.42	15.7	643.4	2.51	unknown *
13	1.43	16.2	1.45	22.0	2.51	1.48	13.6	390.1	1.52	unknown *
14	1.49	7.5	1.51	20.0	2.29	1.54	3.8	228.2	0.89	unknown *
15	1.54	4.2	1.56	12.0	1.37	1.58	0.0	119.2	0.46	unknown *

Table 21 : Showed the standard ascorbic acid in track 2. Total fifteen peaks were found with Rf values ranging from- 0.09 to 1.54.

Figure 34 : HPTLC of EJE extract at 254nm

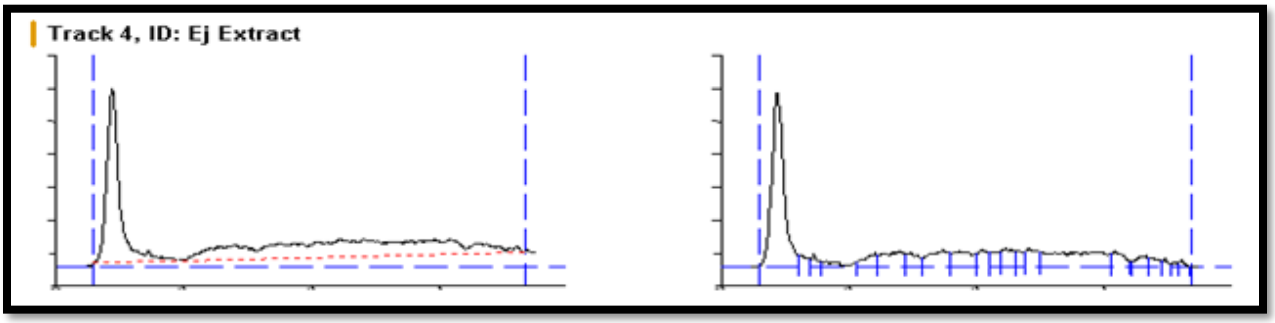


Rf values of EJE extract at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.09	1.1	-0.03	443.5	41.01	0.07	41.7	12693.9	50.78	unknown *
2	0.10	36.8	0.15	52.3	4.84	0.19	39.4	1684.0	6.74	unknown *
3	0.27	43.3	0.29	49.6	4.59	0.33	36.4	1065.6	4.26	unknown *
4	0.33	37.6	0.34	42.7	3.95	0.39	0.0	621.7	2.49	unknown *
5	0.40	1.5	0.43	38.2	3.54	0.45	34.6	502.5	2.01	unknown *
6	0.52	37.4	0.56	56.5	5.22	0.57	50.6	893.7	3.57	unknown *
7	0.57	51.3	0.58	56.9	5.26	0.65	39.5	1618.6	6.47	unknown *
8	0.68	40.9	0.69	45.3	4.19	0.73	24.4	904.2	3.62	unknown *
9	0.74	24.5	0.75	27.5	2.54	0.77	12.2	320.0	1.28	unknown *
10	0.78	12.5	0.81	33.2	3.07	0.82	31.0	507.9	2.03	unknown *
11	0.83	29.1	0.84	37.2	3.44	0.87	27.3	562.2	2.25	unknown *
12	0.88	25.8	0.91	37.6	3.48	0.92	33.7	506.9	2.03	unknown *
13	0.98	16.1	1.05	36.5	3.37	1.07	28.5	1065.6	4.26	unknown *
14	1.07	29.1	1.10	33.1	3.06	1.13	20.2	729.0	2.92	unknown *
15	1.15	12.2	1.17	27.1	2.51	1.18	21.7	255.7	1.02	unknown *
16	1.18	21.7	1.19	24.9	2.31	1.23	8.0	379.6	1.52	unknown *
17	1.23	8.1	1.28	20.2	1.86	1.29	14.5	379.0	1.52	unknown *
18	1.39	3.2	1.44	19.3	1.78	1.45	8.9	310.3	1.24	unknown *

Table 22: illustrated the ethanolic extract of EJ in track 2. Total eighteen peaks were found with Rf values ranging from- 0.09 to 1.39.

Figure 35 : HPTLC of EJE extract at 254nm



Rf values of EJE extract at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.09	1.1	-0.03	443.5	41.01	0.07	41.7	12693.9	50.78	unknown *
2	0.10	36.8	0.15	52.3	4.84	0.19	39.4	1684.0	6.74	unknown *
3	0.27	43.3	0.29	49.6	4.59	0.33	36.4	1065.6	4.26	unknown *
4	0.33	37.6	0.34	42.7	3.95	0.39	0.0	621.7	2.49	unknown *
5	0.40	1.5	0.43	38.2	3.54	0.45	34.6	502.5	2.01	unknown *
6	0.52	37.4	0.56	56.5	5.22	0.57	50.6	893.7	3.57	unknown *
7	0.57	51.3	0.58	56.9	5.26	0.65	39.5	1618.6	6.47	unknown *
8	0.68	40.9	0.69	45.3	4.19	0.73	24.4	904.2	3.62	unknown *
9	0.74	24.5	0.75	27.5	2.54	0.77	12.2	320.0	1.28	unknown *
10	0.78	12.5	0.81	33.2	3.07	0.82	31.0	507.9	2.03	unknown *
11	0.83	29.1	0.84	37.2	3.44	0.87	27.3	562.2	2.25	unknown *
12	0.88	25.8	0.91	37.6	3.48	0.92	33.7	506.9	2.03	unknown *
13	0.98	16.1	1.05	36.5	3.37	1.07	28.5	1065.6	4.26	unknown *
14	1.07	29.1	1.10	33.1	3.06	1.13	20.2	729.0	2.92	unknown *
15	1.15	12.2	1.17	27.1	2.51	1.18	21.7	255.7	1.02	unknown *
16	1.18	21.7	1.19	24.9	2.31	1.23	8.0	379.6	1.52	unknown *
17	1.23	8.1	1.28	20.2	1.86	1.29	14.5	379.0	1.52	unknown *
18	1.39	3.2	1.44	19.3	1.78	1.45	8.9	310.3	1.24	unknown *

Table 23 : illustrated the ethanolic extract of EJ in track 2. Total eighteen peaks were found with Rf values ranging from- 0.09 to 1.39

EC_{50} value of DPPH free radical scavenging of extract

EC50 µg/ml	
Ascorbic acid(standard)	36.13
Eugenia jambolana (extract)	50.78

Table 24 : EC_{50} values of Standard and EJ extract

Increase extract concentration leads to increase antioxidant activity (DPPH scavenging activity) and maximum percentage inhibition was found in the extract has 50 µg/ml concentration. EC50 is a concentration of drug or extract required to obtain a 50% antioxidant effect.

Sample which have EC_{50} lower than 50 µg/ml, is a very strong antioxidant, and 50-100 µg/ml is a strong antioxidant, and 101-150 µg/ml is a medium antioxidant while a weak antioxidant with EC_{50}>150µg/ml. The extract shows a strong antioxidant property with EC_{50} value of 50.78 shown in table 24

7.*8 In vivo* method

Effect of *Eujenia Jambolana* leaves ethanol extract and Rivastigmine on Aluminium chloride induced Alzheimers in rat using Morris water maze. (Escape Latency Time)

Effect of Escape Latency Before And After Administration of Alcl₃

Groups	**Before treatment of $AlCl_3$**	**After treatment with $AlCl_3$**
Control(Normal saline)	016±0.009	0.17±0.008
$AlCl_3$ (17mg/kg)	0.16±0.007	1.29±0.06
EJEI+$AlCl_3$ (17mg/kg+200mg/kg)	0.14±0.008	0.60±0.07
EJEII+$AlCl_3$(17mg/kg+400mg/kg)	0.15±0.003	0.52±0.07
Rivastigmine+$AlCl_3$(17mg/kg +0.3mg/kg)	0.16±0.008	0.50±0.05

Table 25 :Values are mean ± SD. (n=6)

Treatment Effect on Escape Latency By Morris Water Maze

Group	**Treatment**	**Escape latency on post treatment**
1	Control	0.17±0.014
2	$AlCl_3$ (17mg/kg)	1.25±0.17
3	EJEI+$AlCl_3$ (17mg/kg+200mg/kg)	0.52±0.02
4	EJEII+$AlCl_3$(17mg/kg+400 mg/kg)	0.42±0.01
5	Rivastigmine+$AlCl_3$(17mg/ kg +0.3mg/kg))	0.33±0.008

Table 26 : Values are mean ± SD. (n=6). The results is significant at p< .05 ,

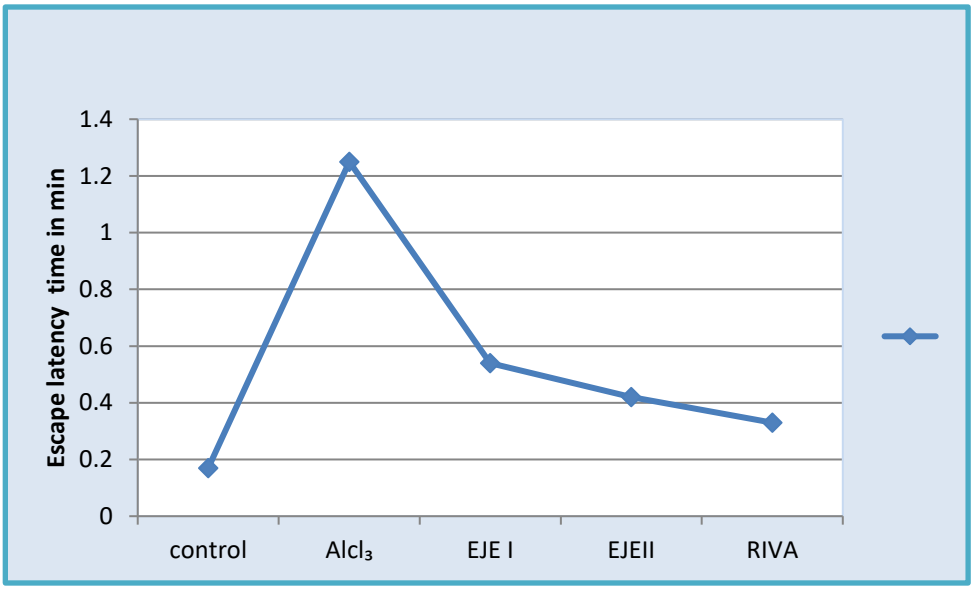


Figure 36 :Graph on treatment effect on morris water maze test

Memory enhancing activity of *Eujenia Jambolana* leaves extract is presented in the table 26 . The escape latency was observed before any treatment and then the escape latency was again observed after 21 days administration of $AlCl_3$ and values are enumerated in table which is significant at $p< .05$ with control group . then after administering the ethanol extract of *Eujenia Jambolana* leaves and standard drug Rivastigmine for 21 days the escape latency was again observed which is like the control do not show much changes in the value (0.17±0.014). The negative control which is $AlCl_3$ treated has value of (1.25±0.17) which is significantly $p<0.05$ increased escape latency to control group. The extract treated group of two doses 200 and 400mg/kg has shown escape latency decreased to the negative control group (0.52±0.02, 0.42±0.01) respectively. The standard Rivastigmine treated group has a marked decrease in the escape latency of(0.33±0.008).The extract and the standard result is significant $p<.05$ to the negative control group.

7.9 Treatment effect on Acetylcholinesterase

Group	Treatment	Acetylcholinesterase activity (µmoles)
1	Control	99±3.39[a]
2	$AlCl_3$ (17mg/kg)	169±4.19[a]
3	Rivastigmine(0.3mg/kg mg/kg)	109±1.15[b]
4	EJE I(200mg/kg)	128±0.77[b]
5	EJE II (400mg/kg)	115±1.12[b]

Table 27 : estimation of acetyl cholinesterse values are mean ±sd (n=6).

[a] indicates p< 0.001 compared to control,

[b] indicates p< 0.001 compared to disease control

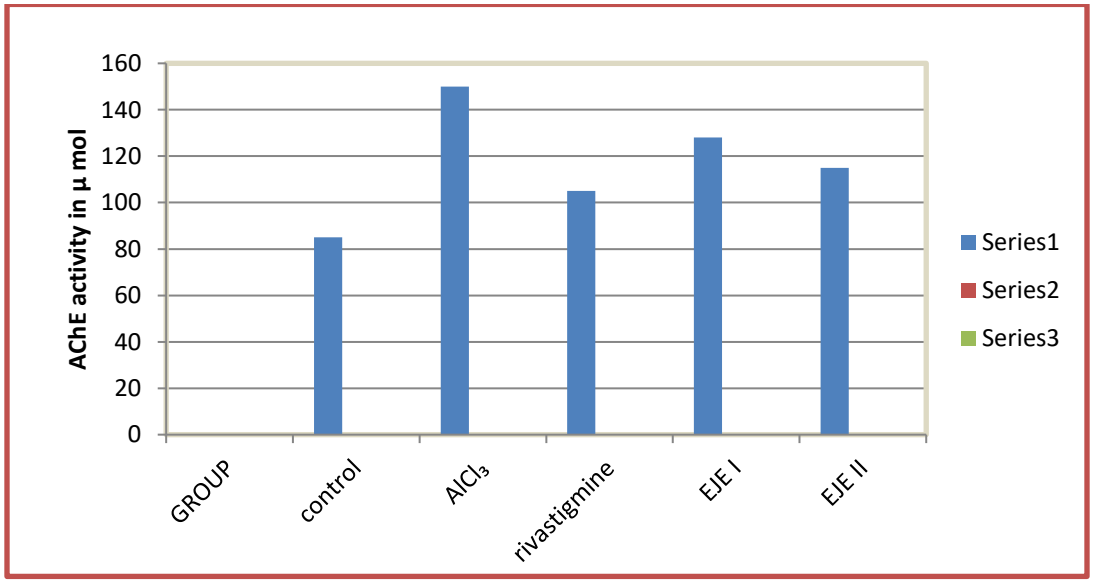


Figure 36 : Graph illustrating the effect of treatment on Anticholinesterase

In this study to determined the level of AChE in the brain homogenate of all group animals, which was used to assess the nootropic activity. . It was observed that administration of $AlCl_3$ resulted in a significantly ($P < 0.001$) increased AChE values (**169±4.19**) as compared to the normal group (**99±3.39**). When the Rivastigmine was administered at the dose of 0.3mg/kg, it has significantly decreased AChE value (**109±0.77**) as compared to the $AlCl_3$ treated group. . The activity of AChE after administration of *Eujenia Jambolana* ethanol extract at the dose of 200mg/kg and 400mg/kg resulted in a significant decreased AChE value (**128±0.77,115±1.12)** as compared to the $AlCl_3$ treated group.

The group treated with 200&400 mg/kg EJE I & EJE II showed decreased AChE activity compared with disease control group. The significance of ($P<0.001$) respectively

8. DISCUSSION

Alzheimer's disease is a genetically heterogeneous neurodegenerative disorder, which is slow in onset but relentless in progress. It is characterized by aphasia, apraxia, and agnosia with the loss of memory as the mine symptoms. The severity and high prevalence of this disease, allopathic system of medicines is yet to provide a satisfactory antidote. Therefore we tried to explore the potential of medicinal plants to manage this deadly disease.

Jamun plant is known to possess diverse phytochemicals, most of which are observed to be of health benefits. The leaves are known to contain β-sitosterol, betulinic acid, mycaminose, crategolic (maslinic) acid, n-hepatcosane, n-nonacosane, n-hentriacontane, noctacosanol, n-triacontanol, n-dotricontanol, quercetin, myricetin, myricitrin and the flavonol glycosides myricetin 3-O-(4″-acetyl)-α-L-rhamnopyranosides, acylated flavonol glycoside.

Eugenia Jambolana leaves were extracted with ethanol using soxhlet extraction method. The phytochemical evaluation for the extract was done.

An extensive docking studies was carried out with polyphenolic ligands Methyl gallate , Myricetrin and Nilocitin with standard ligand as Rivastigmine against protein AChE and BuChE

Insilico studies of polyphenols against standard Rivastigmine was performed against Acetyl cholinesterase and butyrylcholinesterase . Methyl gallate shows similar interaction with the AChE protein with binding energy of(-6.8 kcal mol^{-1}) than Myricitrin (-8.3 kcal mol^{-1}) and Nilocitin (-8.6 kcal mol^{-1}) when compared to standard Rivastigmine with binding energy (-6.3 kcal mol^{-1}) , Methyl gallate shows similar interaction with the BuChE protein with binding energy of(-6.3 kcal mol^{-1}) than Myricitrin (-10.2 kcal mol^{-1}) and Nilocitin (-10.1 kcal mol^{-1}) when compared to standard Rivastigmine with binding energy (-7.1 kcal mol^{-1})

In ADMET studies about absorption methyl gallate is more water soluble ,Myricetrin and Nilocitin has $CaCo_3$ permeability, Nilocitin has greater absorption than standard Rivastigmine.All the compounds are skin permeable , Except Methyl gallate compound the other phytoconstituents are not likely to be a Substrate of P-glycoprotein, Methyl gallate inhibits P-glycoprotein-I while other two compounds are can't be inhibit P-glycoprotein-I like standard

Rivastigmine , from this prediction more over all the Phytoconstituents inhibits P-glycoprotein-II unlikely to standard drug Rivastigmine .

About distribution profile of ligands highly volume of distribution 1.552(log L/kg) in blood plasma compared to standard drug Rivastigmine which has volume of distribution 0.625 (log L/kg) in blood plasma. Except Nilocitin compound while other two phytoconstituents shows greater fraction unbound value in plasma unlikely to both of the ligand Nilocitin has a permeability to cross BBB with a value of -0.008 like standard drug Rivastigmine. About CNS permeability Nilocitin has CNS permeability shows -1.34 lies between the reference value .

About predicted metabolism of ligand expect Nilocitin and the other phytoconstituents are not likely to be metabolized by the substrate of CYP3A4 like Standard drug Rivastigmine which is metabolized by the Substrate of CYP2D6

About excretion profile of ligand all the compounds are considered to have a significant total clearance value, whereas one Phytoconstituents i.e, Methyl gallate shows the greater clearance value of 0.635 than standard Rivastigmine 0.557. All the compounds are not likely to be a Substrate of Organic Cation Transporter 2 (OCT2) that plays an important role in disposition and renal clearance of drugs and endogenous compounds.

Toxicity studies of ligand in ADMET studies was found to be a like compounds are considered not to be a mutagenic and therefore not act as a carcinogen. All maximum tolerated dose was found out . All the compounds are not likely to inhibit hERG I (human ether-a-go-go gene) which are the principal causes for the development of acquire long QT syndrome - leading to fatal ventricular arrhythmia. All compounds are analysed for the toxic potency of a potential compound all have less LD_{50} compared to Rivastigmine. All Phytoconstituents are considered not to be a hepatotoxic, not likely to be associated with skin sensitization, also not with T. Pyriformis toxicity and also not with Minnow toxicity

On estimating drug likeness of the ligand it was found out that Methyl gallate do not possess any violation which can be taken as a oral drug like standard. The Myricetrin and Nilocitin has 3 and 2 violations respectively.

Preliminary phytochemical screening was done on the extracts to identify the presence of various primary and secondary metabolites. There was a presence, metabolites like alkaloid ,flavanoid ,terpinoids , glycosides, tannins

On estimation of total phenolic content in the plant using Folin-Ciocalteu reagent the result was found out be 19.947µg/ml

The antioxidant activity of the extract was performed in HPTLC by estimating the percentage area of inhibition was almost same with the standard Ascorbic acid .The ethanol extracts of two showed areas of activity on high Rf at 1.44 and 1.55 respectively for both applied spots. For the standard ascorbic acid showed areas of activity on high Rf at 1.53 and 1.56. The results for all species remain in good agreement with the data referring to the spectrophotometric DPPH assay method with E_{50} value 50 µg/ml

Aluminum (Al) is a neurotoxin that leads to development of anxiety disorders, depression, memory deficits and symptoms similar to those for AD. Al induces neuronal loss, ultra structural alterations in the different brain regions and biochemical modifications that are implicated in AD development. An elevated amount of deposited Al has been reported in AD brain. Al crosses the blood-brain barrier (BBB) through a specific transferrin receptor (TfR) and induces profound memory loss via disruption of various normal neuronal functions. Al predominantly accumulates in the hippocampus & frontal cortex, regions known to be particularly susceptible in AD. Al supplementation causes neurodegeneration and apoptotic neuronal loss along with cognitive dysfunction, as it is a potent cholinotoxin.

The memory enhancement activity was tested by Morris water maze method in which the escape latency of extract of two dose 200mg and 400mg /kg has taken time of (0.52±0.02, 0.42±0.01) respectively. The standard Rivastigmine treated group has a marked decrease in the escape latency of(0.33±0.008).The extract and the standard result is significant $p<.05$ to the negative control group. So the extract treated group has taken less time like standard group when compared to the negative control Aluminium chloride treated group

On estimating the brain AChE by Ellmans method it was found out that the AChE level of negative control was high value when compared with the standard . The $AlCl_3$ treated has a value

of (169±4.19), and the extract of two doses treated group has a reduced value of (128±0.77), (115±1.12). The standard Rivastigmine treated group has a value of (109±1.15).

9 .CONCLUSION

Eugenia jambolana Lam. (Myrtaceae) is widely used in India to treat several ailments in the traditional system of medicine**.**The present study focused on some use of plant *Eujenia jambolna* which is experimentally proven with some studies like HPTLC, and animal model

The insilico study was performed to study the neuroprotective effect and the mechanism of these plants, phytoconstituents by using proteins AChE and BuChE, for Methyl gallate , Myricetrin,and Nilocitin and Rivastigmine is used as standard .

The central cholinergic system plays an important role in symptoms and sighs of alzheimers . In the present study, *Eujenia jambolna leaves* extracts was administered orally in two doses 200mg and 400 mg/kg to assess the learning and memory. $AlCl_3$ was used to induce AD in rat and the study is performed in model Morris water maze. The study proves that the extract has a neuroprotective action proved by decreasing escape latency morris water model , and has a AChE inhibition action which is proved by Ellman's test. Furthur more studies with isolated bioactives from *Eugenia Jambolana* and making novel drug delivery formulation for Alzheimers

The HPTLC study report confirmed that it contains 7 to 15 phytoconstituents and by using standard Ascorbic Acid the antioxidant activity of the extract was performed by analyzing the % inhibition by analyzing the area inhibited in DPPH scavenging activity which showed neuroprotective action of the extract.

Furthur studies can bring out plant derived drugs with less side effects when compared to existing allopathic drugs.

Further studies in future can be carried out to elucidate the other neurotransmitter to evaluate the mechanism of action, clinical studies may for carried out to establish its efficacy in humans

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ANNEXURE

भारतसरकार
GOVERNMENT OF INDIA
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MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2021/Tech/251

दिनांक/Date: 25.11.2021

पौधे प्रमाणीकरण प्रमाणपत्र /PLANT AUTHENTICATION CERTIFICATE

The plant specimen which has been brought by you for authentication is identified as *Syzygium cumini* (L.) Skeels [= *Myrtus cumini* L.] – MYRTACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

25/11/2021

डॉ.एम. यू. शरीफ़/Dr. M.U. Sharief
वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष/
Scientist 'E' &Head of Office

सेवा में / To

Mrs. Sreya S
Final Year M. Pharmacy
Department of Pharmacology
Karpagam College of Pharmacy
Coimbatore – 641 032, Tamil Nadu

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
(Reg.No: 1762/PO/Re/S/14/CPCSEA)
KARPAGAM FACULTY OF MEDICAL SCIENCES RESEARCH
COIMBATORE- 641 032, TAMILNADU, INDIA

Date:10/12/2021

CERTIFICATE

This is to certify that the project title **"Evaluation of neuroprotective effect of isolated polyphenolic compound from leaf of eugenia jambolana for anticholinesterase and antioxidant activity"** has been approved by the IAEC.

IAEC NO - KFMSR/M. Pharm/03/2021

Name of Chairman/Member Secretary IAEC: T.K.Pon 10/12/21
[Dr. T.K. Ponnuswamy]

Name of CPCSEA nominee: 10/12/2021.
Dr. C. Ganeshkaran

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This is to certify that

Sreya.S

has participated in the International webinar on "Recent Advances in Preclinical Research" organized by Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, INDIA in association with SERB (Science and Engineering Research Board), held on 06th JUNE 2020.

Sri.Y.V Anjaneyulu
President
Chalapathi Educational Society

Prof.Rama Rao Nadendla
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CERTIFICATE OF PARTICIPATION

This certificate is presented to

Sreya.S

for participated as a delegate in a Webinar held on 09th October 2020 entitled on "Scope of Clinical Research and Associated Domains" Organised by Department of Pharmacology.

IN ASSOCIATION WITH
INDIAN PHARMACEUTICAL ASSOCIATION
COIMBATORE LOCAL BRANCH

Dr. C. SENTHIL KUMAR
Professor & Head
Dept.
Pharmacology

Dr. S. MOHAN
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