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DECLARATION

I, Shipra Rathour, 2K21/MSCBIO/47 of MSc. Biotechnology, hereby declare that the project Dissertation titled "Evaluation of *Capparis Decidua*'s potential as an alternative approach for AD: A gene enrichment and molecular docking study." which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science is authentic record of my own carried out work under the supervision of professor Yasha Hasija. The matter presented in this report has not been submitted by me for the award of any other degree of this or any other Institute/University. The work has been accepted in IEEE conference with the following details:

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Place: Delhi

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CERTIFICATE

I hereby certify that the Project Dissertation titled "Evaluation of *Capparis Decidua*'s potential as an alternative approach for AD: A gene enrichment and molecular docking study." which is submitted by Shipra Rathour, 2K21/MSCBIO/47, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Alzheimer's disease (AD) is a long-term neurodegenerative condition marked by memory loss and cognitive deterioration. The diagnosis of AD is further supported by the presence of recognisable lesions including neuritic plaques, neurofibrillary tangles (NFTs), cerebral amyloid angiopathy, neuronal loss, and cholinergic insufficiency. As a result, therapeutic intervention may be possible by targeting critical proteins linked to the condition, such as the enzyme that cleaves amyloid precursor protein and cholinesterases like acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Despite continued research, there is still no cure for AD, hence it is necessary to investigate bioactive substances obtained from natural sources to improve existing therapy strategies.

A medicinal plant from the Capparacea family called Capparis decidua has a wide variety of secondary metabolites, including terpenoids, alkaloids, polyphenols, and phytosterols. These substances have a number of advantageous qualities, such as antioxidant, anthelmintic, anti-diabetic, and anti-inflammatory actions. This pilot study intends to investigate the potential of bioactive chemicals produced from Capparis decidua as possible options for treating AD in light of these qualities. To accomplish this, a preliminary in-silico analysis was carried out, particularly concentrating on the therapeutic efficacy of Capparis decidua in reducing AD. 55 phytochemicals from Capparis decidua were used in the investigation to determine their binding affinities to the target protein acetylcholinesterase. In order to shed light on the dysregulated genes linked to AD, gene enrichment analysis was also carried out, which improved our comprehension of the disease processes and possible treatment targets. The results of this study show potential for the creation of new AD treatment approaches. There may be a chance to slow the spread of the illness by using the therapeutic benefits of Capparis decidua and concentrating on AChE. However, in order to evaluate the safety and effectiveness of the discovered bioactive compounds, it is essential to confirm these in-silico results through further in vitro and in vivo research.[20]

This pilot investigation concludes by highlighting the potential of bioactive substances obtained from Capparis decidua as prospective therapy options for AD. To clarify the underlying processes and progress the creation of efficient treatments for this crippling condition, more research, including gene enrichment analysis and experimental confirmation, is required.

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LIST OF ABBREVIATIONS

1. AD – Alzheimer's Disease
2. Ach- Acetylcholine
3. AChE - Acetylcholinesterase
4. BuChE. - Butrylcholinesterase
5. RO5- Rule of five
6. NRL- Nuclear receptor ligand
7. ICM- Ion Channel Modulator
8. GPCR: G-protein coupled Receptor
9. EI- Enzyme Inhibitor
10. KI- Kinase Inhibitor
11. BBB- Blood Brain Barrier
12. AChEI - Acetylcholinesterase Inhibitors
13. BACE-1- β -site precursor protein cleaving enzyme-1
14. FDA- Food and Drug Administration
15. SAR- Structure activity relationship
16. NFT - Neurofibrillary tangles
17. APP- Amyloid precursor protein
18. NMDA- N-Methyl-D-aspartate Receptor
19. MAO – Monoamine oxidase
20. AEP: Aspargine endopeptidase
21. AIF- Apoptosis inducing factor
22. PARP- poly(ADP-ribose) polymerase-1
23. ChAT- Choline acetyltransferase
24. Acetyl-Co A- Acetyl coenzyme A
25. VChAT- Vesicular acetylcholine transporter
26. CHT1- Choline transporter
27. BBB- Blood Brain Barrier
28. PDB- Protein Database
29. IMPPAT- Indian Medicinal Plants, Phytochemicals and Therapeutics
30. ML- Machine Learning
31. MD- Molecular Dynamics
32. PLIP- Protein Ligand Interaction Profiler
33. ADME- Absorption, Distribution, Metabolism, Excretion
34. SMILES- Simplifies Molecular Input Line Entry System

CHAPTER 1: INTRODUCTION

Dementia can develop over time as a result of a variety of illnesses known as neurodegenerative disorders, which affect the structure and function of nerve cells. The age-related neurological ailment with the highest prevalence among them is Alzheimer's.

Both the buildup of phosphorylated Tau protein, which results in neurofibrillary tangles intracellularly, and the accumulation of amyloid- (A) peptides, which form neuritic plaques extracellularly, contribute to the pathogenesis of the illness. The cholinergic theory is the earliest and most thoroughly investigated of the several hypothesised aetiologies and pathogeneses.

Finding genes that may contribute to the development of Alzheimer's disease due to their dysfunctional behaviour may be done effectively using gene enrichment analysis. Our research showed that the AChE gene showed upregulation, contributing to the initiation and development of this illness. To stop the functioning of this gene, we'll employ a technique called molecular docking. By using a computational method, we can investigate how chemicals interact, which will help us find new substances or medications that can specifically target and block the function of the AChE gene. These therapies provide hope for therapeutic improvements in the treatment of Alzheimer's.

According to the theory, the neurotransmitter acetylcholine (Ach) is crucial for memory formation, synaptic transmission, and neuronal excitement. With the aid of a group of esterase, including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), the acetylcholine in the synapse is converted into acetyl and choline. The BACE-1 enzyme, insufficient signalling, and the -site precursor protein cleaving enzyme, aberrant synaptic transmission are additional important components in the pathogenesis of AD. Although there are FDA-approved treatments for AD, they only prevent the illness from starting.

As a viable alternative strategy to inhibit acetylcholinesterase (AChE) and so lessen AD symptoms, the goal of this work is to conduct an in-silico examination of phytochemicals derived from Capparis decidua. The Capparaceae family's tropical and subtropical plant Capparis decidua possesses outstanding medicinal qualities. Alkaloids, polyphenols, terpenoids, and fatty acids are only a few of the phytochemical components that are responsible

for its medicinal properties. Due to their accessibility and simplicity of extraction, powerful pharmacological characteristics, and low toxicity, phytochemicals produced from natural sources have become more and more popular as unconventional medicinal agents.

Utilising Autodock Vina, 55 phytochemicals are recovered from Capparis decidua. The selection criteria were based on the phytochemicals' affinity for binding, which was compared to that of donepezil, an FDA-approved common AChE inhibitor used to treat AD. Following this, the chosen phytochemicals were tested and evaluated for their drug-likeness, bioactivity, bioavailability, and compliance with Lipinski's RO5. The goal of this review is to find innovative prospective compounds for AD target intervention, overcome the shortcomings of current therapy methods, and eventually enhance the currently used management techniques for AD.

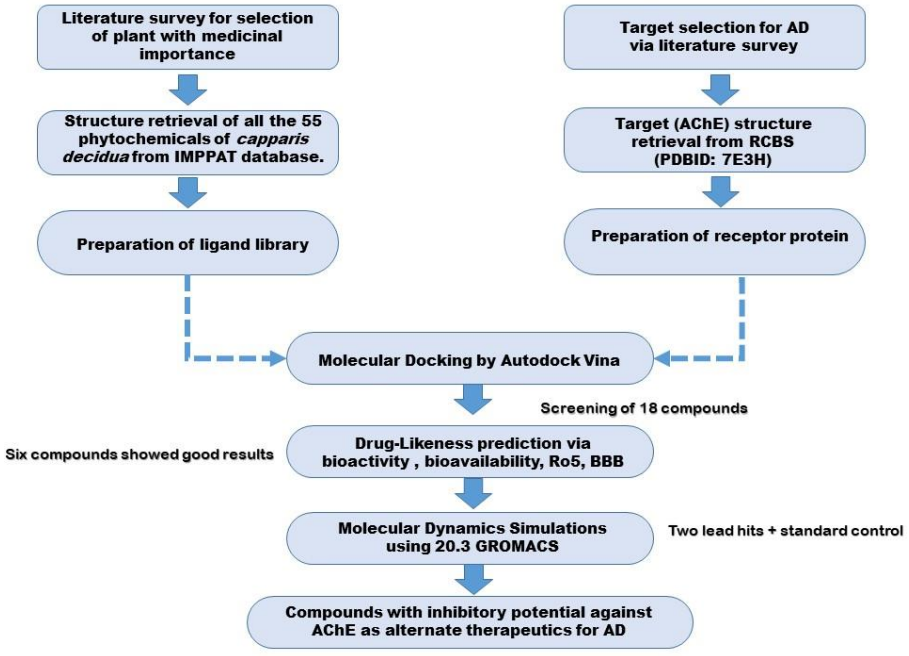

Fig.1: Overview of workflow of Methodology.

CHAPTER 2: LITERATURE REVIEW

2.1 General

Disorders known as neurodegenerative illnesses slowly erode the nervous system's composition and efficiency. They include illnesses including ALS, Parkinson's, Huntington's, and Alzheimer's. These conditions cause nerve cells to gradually deteriorate, impairing cognition, movement, and other neurological processes. Numerous variables, such as genetics, protein misfolding, and inflammation, are their underlying causes. Unfortunately, there are few effective treatments available. Ongoing research is working to better understand the processes at work and create drugs that might reduce or stop disease development.

2.2 Overview of Alzheimer's

One of the most difficult and expensive diseases of the contemporary period is Alzheimer's disease, a significant type of dementia. All throughout the world, it has a serious detrimental effect on people, families, and healthcare systems. Millions of people are dramatically impacted by Alzheimer's disease, which causes memory loss, behavioural abnormalities, and gradual cognitive decline. The frequency of this neurodegenerative disease is predicted to increase as life expectancy increases, highlighting the need for continued research and novel approaches to address prevention, early identification, and efficient treatment interventions.[3]

The World Alzheimer Report estimates that in 2015, dementia impacted more than 46.8 million people globally. It is projected that this number will nearly double every 20 years. Surprisingly, there are said to be 55 million people living with dementia worldwide, with a new case reportedly occurring every three seconds. Notably, 60 percent of these instances are discovered in developing and middle-income nations. These figures demonstrate the significant worldwide effect of dementia.

Alzheimer's disease symptoms include behavioural problems, cognitive decline, and memory loss. Risk factors include a person's age, inheritance, family history, elevated blood pressure, obesity, or diabetes, and certain lifestyle choices, yet the specific reason is still unknown.

Extracellular Amyloid Plaques, or "A Plaques" or "senile plaques," are characterised by the accumulation of amyloid-(A) peptides, whereas intracellular neurofibrillary tangles (NFTs) are predominantly made up of tau-rich proteins. The A plaques and NFTs are two well-known pathological characteristics these are important in the development and progression of Alzheimer's disease and serve as the primary indicators of the condition.[5]

2.3 Pathogenesis

The aberrant phosphorylation and aggregation of tau proteins is the main cause for neurofibrillary tangles (NFTs) to develop. Memory deficits and neuronal death result from this process, which also affects axonal transport and the integrity of microtubules. These occurrences underscore the importance of abnormal tau proteins in the pathophysiology of the illness and indicate a relationship between them and the cognitive loss seen in Alzheimer's disease.

In the past, it was widely believed that the accumulation of A peptides was primarily responsible for the aberrant how tau protein functions in Alzheimer's illness. However, recent studies suggest that both processes can take place simultaneously, increasing their detrimental effects and adding to the cognitive decline seen in the condition. This emphasises the complex interrelationship between tau pathology and A deposition, underlining their combined contribution to Alzheimer's disease development.

Numerous physiological processes are involved in the removal of A from the brain. These include the breakdown of A by peptidase enzymes like insulin-degrading enzyme, endothelin-converting enzyme, and neprilysin. Arachnoid villi, the glymphatic-lymphatic system, the blood-brain barrier, interstitial fluid bulk flow, and others are only a few of the processes. via which A can be delivered to the peripheral circulation. Additionally, astrocytes, perivascular macrophages, and microglia take involved in the phagocytosis and destruction of A. A decrease in A clearance may result from any impairment in these mechanisms[4]. The buildup of A-peptides brought on by this decreased clearance, when coupled with increased A synthesis, might eventually cause neuronal dysfunction and degeneration. It is thought that the existence of plaques in Alzheimer's disease causes a reservoir of soluble A oligomers and plays a role in their sequestration. Numerous studies have documented the buildup of these soluble oligomers in Alzheimer's patients' brains, notably in the synaptic areas of those patients exhibiting memory loss. These oligomers help dementia develop by causing abnormal alterations in synaptic structures. It's crucial to remember that synaptic disruption is a factor in cognitive impairment in Alzheimer's disease in addition to the buildup of aberrant tau and A proteins. This emphasises the importance of broken synaptic connections and decreased neural transmission in the growth of Alzheimer's disease. Our knowledge of the illness and the creation of successful intervention and therapy techniques depend heavily on the interaction between accumulation and synaptic dysfunction.

It has been found that A peptides may attach to cell receptors and start a variety of calcium and oxidative signalling cascades. These peptides weaken synaptic connections between neurons by interfering with synaptic plasticity receptors, increasing the release of glutamate neurotransmitters, promoting tau hyperphosphorylation, disrupting axonal transport, and contributing to memory impairment and inhibition of long-term potentiation (LTP).

The specific mechanisms at play are still unclear despite substantial investigation into the processes underpinning the toxicity of A peptides. N-methyl-D-aspartate (NMDA), butyrylcholinesterase (BuChE), and acetylcholinesterase receptors, beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), asparagine endopeptidase (AEP), monoamine oxidases (MAO), and protein kinases are just a few of the proteins linked to the neurological dysfunction in Alzheimer's disease (AD). These proteins are crucial for cholinergic neurotransmission, synaptic activity, processing of amyloid precursor proteins, protein degradation, and cellular signalling, among other functions. For understanding the underlying causes of AD and creating specialised treatment plans, it is essential to comprehend the intricate interactions between these proteins and A peptides.[7,8]

Additionally, research in the literature indicate that excessive PARP-1 activity brought on by cellular damage or oxidative DNA damage results in the consumption of a lot of NAD+ and ATP. By activating apoptosis-inducing factor (AIF), this eventually encourages the death of neuronal cells by parthanatos. Therefore, it has been suggested that inhibiting PARP-1 as a viable alternative therapy method for Alzheimer's disease (AD).

2.3 Gene Enrichment Analysis

It is a technique for learning about a group of genes' biological importance. It enables us to determine whether certain gene sets or metabolic pathways are more common in the provided gene list than would be predicted by chance. The gene list may be compared to databases and current information to find biological themes, functional groups, and molecular pathways that might be overrepresented.

The AChE gene showed an overexpression in our study, indicating higher expression levels. We carried out a gene enrichment study to obtain understanding of its biological consequences. This computational method finds pathways and enriched gene sets connected to the elevated gene.

The elevated AChE gene was the subject of substantial findings from gene enrichment analysis. It was discovered to be linked to enhanced biological processes, such as synaptic plasticity, cholinergic neurotransmission, and neurodevelopment. Significant enrichment was also seen in the molecular pathways involved in synaptic transmission, neurotrophin signalling, and acetylcholine signalling. We will be able to comprehend the consequences of AChE overexpression and the underlying molecular pathways as a result. and pathways that this gene influences and which lead to AD[9]. Additionally, it could advance our knowledge of physiological processes that occur naturally and provide information on pathological disorders that involve dysregulated cholinergic signalling.

The AChE gene has been linked to Alzheimer's disease through upregulation. We will employ molecular docking, which is a computer method that evaluates interactions between tiny compounds and the AChE protein, to limit its activity.

2.4 AChE: The key Target

With almost 1500 scholarly publications indexed in PubMed, AChE has been the subject of substantial research regarding Alzheimer's illness. The majority of these research concentrate on AChE-I inhibitor-based therapeutic strategies. These studies explore the use of AChE-I as prospective treatment approaches for Alzheimer's, underscoring the importance placed on comprehending the function of AChE and its inhibition in treating the condition.[19]

Acetylcholine (ACh) is essential for the formation of memories, synaptic transmission, and neuronal excitability in the nervous system. Acylcholinesterase (AChE) and butyrylcholinesterase (BuChE), two enzymes,, help break down acetylcholine into choline and the corresponding acid. AD, which is brought on by the death of neurons in the hippocampus region of the brain, is characterised by both a deficiency in acetylcholine and a decrease in cholinergic neurons. This chemical imbalance is what causes the cognitive and memory impairments found in AD patients. Acetylcholine (ACh), a neurotransmitter that serves as a chemical messenger to promote communication between neurons, is essential to the cholinergic system. By converting ACh into acetate and choline through the process of hydrolysis, AChE fulfils a key role in the body. By successfully stopping neurotransmission at synapses, this mechanism prevents overstimulation and preserves the delicate balance of neuronal signalling.

Despite certain similarities, it's crucial to remember that butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) serve different purposes. BChE can hydrolyze butyrylcholine and functions as a defence mechanism against organophosphate nerve agents, whereas AChE largely controls acetylcholine levels[26]. Together, these enzymes contribute greatly to the upkeep of typical brain function by playing crucial roles in the regulation and preservation of cholinergic neurotransmission.

A complex between Amyloid and AchE forms when the normal assembly of A peptides is interfered with by the unbalanced amounts of AchE. This complex contributes to the underlying processes of the disease and plays a crucial role in the onset and development of Alzheimer's disease.

As an alternative to AChE, BuChE is a non-selective cholinesterase that can degrade both butyrylcholine and acetylcholine as well as acetylcholine. AChE is recognised for its high catalytic activity in the degradation of acetylcholine (80%).

2.5 Potential alternatives include plant-derived natural products.

Natural products have the potential to be safe and effective pharmacodynamic agents for treating neurodegenerative disorders. However, the complexity of afflicted organs like the brain and their aggressive character, in addition to the numerous biochemical pathways and proteins implicated in the pathogenesis of these disorders, represent considerable hurdles in the development of treatments for Alzheimer's disease.[12]

Natural products made from plants and their bioactive compounds have recently been the focus of extensive inquiry., with an emphasis on their possible therapeutic uses in neurodegenerative illnesses like Alzheimer's disease (AD). Despite the fact that the efficacy of phytomedicines is still not fully understood, they are becoming more and more accepted as complementary and alternative therapies due to their affordability, decreased toxicity, widespread availability, and few side effects. The goal of this expanding body of research on phytochemicals is to examine their bioactivity and nutritional importance across various component groups. The traditional usage of plants as medicines and the discovery of molecular building blocks for contemporary medications inside plants emphasise the significance of studying these natural chemicals.[12]

The hardy, densely branched climbing shrub Capparis decidua Edgew (Forssk.), sometimes known as C. aphylla, is a member of the Capparidaceae family. It flourishes in tropical and

subtropical climates, which are home to it naturally. There are several regional names for this plant, including Karil, Kabra, Hanbag, Kair, Delha, Caper, Karyal, and others. It has historically been used in traditional medicine to treat conditions including rheumatism, asthma, cough, lumbago, pyorrhoea, dysentery, liver infections, fevers, heart problems, constipation, ulcers, piles, renal abnormalities, and skin illnesses.. Its popularity in conventional therapeutic methods has been influenced by its adaptability in treating a variety of illnesses.

The sturdy and densely branched climbing shrub Capparis decidua, commonly known as C. aphylla, is a member of the Capparidaceae family. It has a variety of regional names, including Kari, Delha, Caper, Kair, Karil, Kabra, and others. It flourishes in subtropical and tropical climates. This plant has a long history in traditional medicine and has been used to treat a variety of illnesses, including rheumatism, asthma, cough, toothache, liver infections, fevers, constipation, ulcers, and skin conditions.[15,16] Its prestigious reputation is a result of its several beneficial characteristics as a carminative, aphrodisiac, analgesic, and antioxidant. Beyond its therapeutic relevance, C. decidua also has antibacterial, antifungal, neuroprotective, and antigout effects, making it highly regarded.

Sitosterols, a class of plant sterols with a reputation for having possible health advantages, have been found in Capparis decidua, according to research. These substances have antioxidant characteristics that efficiently combat the body's dangerous free radicals. Additionally, C. decidua includes polyamines, aliphatic components, diterpenic ester, and diterpene alcohol, all of which contribute to its medicinal qualities. Additionally, the bioactive substances discovered in C. decidua are essential for the growth, development, and proliferation of mammalian cells, suggesting that they may be a lot of biological processes are involved. These findings demonstrate the importance of C. decidua in both conventional and modern medicine and point to its hopeful future in research and healthcare.

2.6 Molecular Docking

The molecular docking study is computationally driven research designed to assess any generated candidate's efficacy at an early stage, specifically targeting any disease-associated

target. Currently, the majority of researchers pick "hit" or "lead" candidates using sophisticated computational algorithms.

A computer technique called molecular docking is used to forecast the interactions that will occur when a tiny molecule (or ligand) binds to a target protein. By evaluating the binding affinity and manner of possible drug candidates inside the target protein's active site, it seeks to discover and optimise these possibilities.[18] The development of stable complexes is predicted by molecular docking by modelling the three-dimensional structures of the ligand and protein. Lower scores correspond to stronger binding, and scoring functions are assigned to the bound ligands depending on their binding affinities. This method is essential in the early stages of drug development because it helps identify promising compounds, improves the chemical structures of those compounds, and directs additional experimental research.

The acetylcholinesterase (AChE) protein was the main subject of our inquiry because it didn't show any mutation-related changes. This protein's Protein Data Bank (PDB) reference code was determined to be 7E3H. We used the crystallised structure of human AChE as our comparison control since it combined with the FDA-approved medication Donepezil to produce a complex.

Chain A and Chain B, the two separate chains that make up the 7E3H protein structure, are essential to the protein's functionality. With 540 amino acids, the acetylcholinesterase (AChE) molecule has a unique shape and set of abilities. As part of our research, we also looked at a ligand known as E20, which combined with chains E and F to form a complex. The total structure had a significant weight of 120.37 kDa, highlighting its size and intricacy.

The most prevalent softwares utilised for molecular docking include Molegro Virtual Docker, FlexX, DockThor, GOLD, AutoDock and AutoDock Vina. This study employed Autodock Vina and Autodock Perl as they offer rapid as well as precise analysis. The 3D structures of ligands to be screened can be retrieved from databases like PubChem, ZINC and PDB.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Preperation of Dataset

The NCBI Gene Expression Omnibus (GEO) database was used to get the RNA-sequenced dataset used in this investigation. The collection included tissue samples obtained from people with Alzheimer's disease (AD) and those who were not afflicted, allowing comparisons between the two groups.

Table 1: Brief information on the selected dataset.

Tissue Origin	**Geo Accession number**	**Sequencing technique**	**Sample size**
Brain Tissues	GSE138260	RNA sequencing OR High throughout sequencing	19 - Control 17 - Affected

(A)

Sample_geo_accession	**Sample Title**	**Disease state**	**Gender**
GSM4103849	Brain_AD_sample1	AD	Female
GSM4103850	Brain_AD_sample3	AD	Male
GSM4103851	Brain_AD_sample4	AD	Female
GSM4103852	Brain_AD_sample5	AD	Female
GSM4103853	Brain_AD_sample6	AD	Female
GSM4103854	Brain_AD_sample7	AD	Female
GSM4103855	Brain_AD_sample8	AD	Female
GSM4103856	Brain_AD_sample9	AD	Female
GSM4103857	Brain_AD_sample10	AD	Male
GSM4103858	Brain_AD_sample11	AD	Male
GSM4103859	Brain_AD_sample12	AD	Male
GSM4103860	Brain_AD_sample13	AD	Female
GSM4103861	Brain_AD_sample14	AD	Male

GSM4103862	Brain_AD_sample15	AD	Female
GSM4103863	Brain_AD_sample16	AD	Male
GSM4103864	Brain_AD_sample17	AD	Male
GSM4103865	Brain_AD_sample18	AD	Female
GSM4103866	Brain_control_sample1	Control	Male
GSM4103867	Brain_control_sample2	Control	Male
GSM4103868	Brain_control_sample3	Control	Female
GSM4103869	Brain_control_sample4	Control	Male
GSM4103870	Brain_control_sample5	Control	Male
GSM4103871	Brain_control_sample6	Control	Female
GSM4103872	Brain_control_sample7	Control	Male
GSM4103873	Brain_control_sample8	Control	Female
GSM4103874	Brain_control_sample9	Control	Female
GSM4103875	Brain_control_sample10	Control	Female
GSM4103876	Brain_control_sample11	Control	Female
GSM4103877	Brain_control_sample12	Control	Female
GSM4103878	Brain_control_sample13	Control	Female
GSM4103879	Brain_control_sample15	Control	Male
GSM4103880	Brain_control_sample16	Control	Male
GSM4103881	Brain_control_sample17	Control	Male
GSM4103882	Brain_control_sample18	Control	NA
GSM4103883	Brain_control_sample19	Control	Female
GSM4103884	Brain_control_sample20	Control	Male

(B)

3.2 Construction of a Dataset Notebook

The GEO programme was used to analyse the raw RNA-sequencing dataset, producing a standardised and interactive report as a result. A table identifying differentially expressed genes was derived from this investigation. Using updated p-value criteria and log fold change (log Fc) filtering, the gene list was further refined. Genes were specifically categorised as upregulated or downregulated based on whether their logFC value was more than or equal to "2" or less than or equal to "-2." In order to ensure statistical significance in the gene selection, a maximum threshold of "0.05" was also established for the adjusted p-value.

3.3 Exploring plant-based Phytochemicals.

Finding plant phytochemicals whose genes match the dataset was the next goal. The search for phytochemicals produced from plants was made easier by the IMPPAT database. It was predicted after a comprehensive assessment of the literature that the Capparis Decidua plant would have pertinent features, making it a reference in our study.

3.4 Target Finding

We used the Swiss Target Prediction online service to determine the most similar genes, proteins, and small molecule targets. Using this approach, we employed the plant Capparis Decidua in our study to pinpoint possible gene/protein targets that closely resemble the chosen small compounds.

3.5 Graphical the observations for comparing the genes

A variety of graphical representations, including the Volcano graph, Box plot, and Mean Difference plot, provide crucial insights into the expression patterns of genes associated to Alzheimer's disease (AD) after analysing and extracting gene samples from the Gene Expression Omnibus (GEO) database. In this complicated situation, our visual tools make it straightforward to compare overexpressed and underexpressed genes. They provide a greater knowledge of the changes in gene expression in AD by effectively illuminating the differential regulation of genes.

3.6 Selecting and refining receptor proteins.

Finding the gene that codes for our target protein, AChE (acetylcholinesterase), was made possible via the RCBS Protein Database, a very useful web resource. We produced the crystallised form of human AChE coupled to its ligand E20 (CID 3152), which had substantial significance for our study and provided insight into the complex structure and interactions of this protein. We easily got this exact protein structure from databank for proteins (PDB) and were provided with it in the well-known PDB format. Its PDB ID is 7E3H.[] Finding the gene that causes encod was made possible by the RCBS Protein Database, a very useful web resource.For some activities in our investigation, the BIOVIA Discovery Studio (DS Visualizer Client Windows 64 bit) programme was used. These duties involved injecting polar hydrogen atoms while concurrently eliminating water molecules, heteroatoms, and ligands. In addition, we identified the coordinates of the centre GridBox as -43.368 (x-coordinate), 37.722 (y-

coordinate), and -30.313 (z-coordinate) using the SBD site sphere and the corresponding ligand in the complex. These exact coordinates were essential to our further studies and research.

Overall, the BIOVIA Discovery Studio software's implementation really benefited our research by enabling crucial modifications and delivering crucial data. The protein that had been produced was stored in PDB (Protein Data Bank) format. Then, Kollman's charge was introduced and distributed using the Autodock Vina 1.7.5 programme to guarantee the accuracy of electrostatic characteristics. Individual atoms were given AD4 kinds, enabling accurate characterisation. Finally, the protein structure was optimised for later docking experiments by converting it from the PDB format to the PDBQT (Protein Data Bank, Quaternary Structure) format. These consecutive stages made it possible to analyse the protein's binding properties and interactions in a more thorough and dependable manner.

3.7 Preparation of phytochemical as ligand library

Donepezil (ZINC597013), a common inhibitor, was obtained in ZINC database in SDF (Structure-Data File) format. The Open Babel GUI was employed to convert the SDF file into PDBQT (Protein Data Bank, Quaternary Structure) format to ensure compliance with our analysis[43]. In addition, the IMPPAT database yielded a library of 55 phytochemicals extracted from Capparis decidua[57]. Using the Open Babel GUI, each phytochemical was separately translated to PDBQT format. We were able to precisely measure and compare the binding characteristics of the inhibitor and the phytochemicals in our investigation thanks to the uniform file formats made possible by these format changes.

3.8 Molecular Docking and Protein Ligand Docking using Autodock Vina

We used Autodock Vina 1.7.5 to do out in-silico molecular docking. Docking required setting up the programme with particular settings. The sizes in the X, Y, and Z dimensions were set to 100, 100, and 110, respectively, while the centre grid coordinates were set to X = -43.368, Y = 37.722, and Z = -30.313. These grid settings were incorporated into a configuration file. A num mode number of 10 was used in the docking settings to provide numerous binding modes, and a value of 4 was used to describe the appropriate energy range for the docking outcomes. All of the ligands, including the common inhibitor and phytochemicals, were docked against the target protein AChE using Autodock Vina. We determined the binding affinities of the ligands

using PERL in Kcal/mol. Important details on the potency of their binds and interactions with the AChE protein were revealed by this analysis.

3.9 Docking Interaction analysis

Using PyMol, PLIP, and BIOVIA Discovery Studio, we reviewed the downloaded output files and log files to assess the results.[35] We were able to correctly identify and pinpoint the specific amino acid residue in the protein structure where the ligand binds by using these methods. To assess the binding affinities of the conventional inhibitor Donepezil and the phytochemical ligands, we conducted a comparative investigation of the free binding energy. The phytochemicals with the lowest binding energies, which are more apt to bind to the target protein, were the ones we were interested in finding. These chosen phytochemicals were then thoroughly evaluated for their pharmacokinetic characteristics. We were able to prioritise and further research phytochemical compounds with promising binding affinities thanks to our methodical methodology, potentially presenting chances for medication development and improvement.

3.10 Virtual screening of pharmacokinetics and Drug likeness of selected phytochemicals

For further investigation, phytochemicals with binding energies comparable to those of the conventional inhibitor, donepezil, were found. These phytochemicals showed comparable binding affinities, suggesting that they could work well as Donepezil supplements or substitutes. They were chosen to go through extra testing to determine their eligibility and possible advantages over the typical inhibitor.

A. **Lipinski's RO5 analysis:** With a maximum of one variation allowed among the five inclusion criteria, the Rule of Five (RO5) analysis establishes specified criteria for orally active drugs. A LogP value of less than 5 and a molecular mass of fewer than 500 daltons and a molar refractive range of 40–130 are some examples of these requirements. Respecting these recommendations assures that oral medication delivery and overall pharmacokinetic profiles will be favourable. The PDB files of the chosen phytochemicals were uploaded to an internet application and exposed to Lipinski's RO5 in order to evaluate their oral action. In addition to the prerequisites indicated above, Lipinski's Rule stipulates that there should be less than five and ten hydrogen bond acceptors, respectively. This research considers a number of variables, such as molecular mass, LogP value, molar refractivity, and hydrogen bonding capacities, to shed light on the phytochemicals' potential oral action.

B. **In-silico Bioavailability analysis:** It's crucial to identify whether a medicine has effectively reached its intended biological target in order to assess its functional efficacy. Understanding the drug's pharmacokinetic features, which include concepts like absorption, distribution, metabolism, and excretion (ADME), is vital to achieving this. We used web-based tools like SwissADME and admetSAR to analyse these features using the canonical SMILES representations of the chosen phytochemicals. With the use of these methods, we were able to analyse and make predictions about key ADME parameters, which gave us vital new information on the drug-like properties and potential therapeutic value of the phytochemicals.

C. **Bioactivity score analysis**: Designing functional drugs that display increased binding selectivity and reduce side effects requires gaining knowledge about the drug's binding cascade. Drugs' scores as nuclear receptor ligands (NRL), kinase inhibitors (KI), enzyme inhibitors (EI), GPCR ligands along with ion channel modulators (ICM), and protease inhibitors (PI) are taken into consideration when evaluating their drug ability as ligands[46]. These results are important markers of how the medicine may interact with particular targets involved in biological processes. We can find ligands with qualities that are advantageous for the creation of therapeutics by examining these scores. The bioactivity scores of selected phytochemicals were predicted using the Molinspiration online tool by providing their canonical SMILES as input data

CHAPTER 4: RESULTS

4.1 Differentially expressed genes identification

We started by carefully choosing a suitable RNA-seq dataset from the GEO database and tailoring it to satisfy our particular requirements. Then, we created an analysis notebook that concentrated on the contrast between control and afflicted samples. The notebook had sections for importing datasets, analysing library sizes, plotting volcanoes, and listing differential expressions. After finishing, we exported the notebook and added further filtering procedures to enhance the outcome[39] The Volcano Plot graph (Fig. 2) was used to graphically show the downregulated genes are shown by blue marks, whereas upregulated genes are denoted by red marks. and it was used to generate a brief summary of the dataset's most important findings.

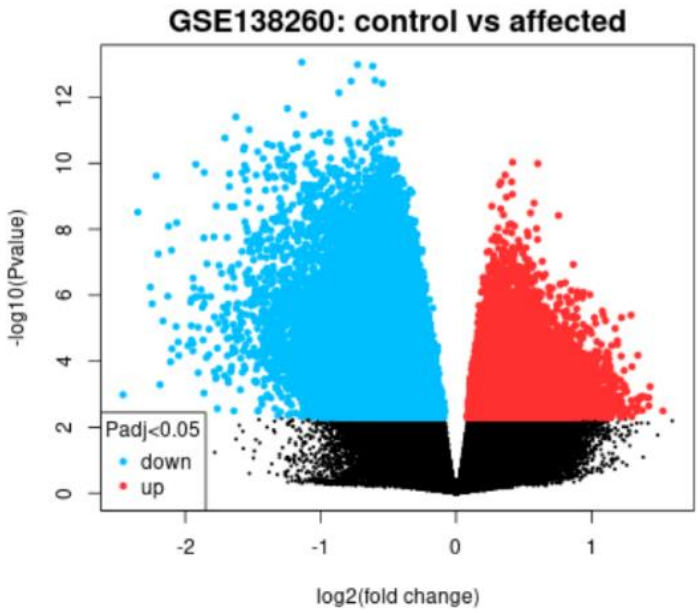

Fig 2: Volcano Plot

Analysis of AD gene expression using a volcano plot reveals significant alterations in inflammatory responses, emphasising their function in the illness. Insights into the molecular underpinnings underlying the pathophysiology of AD provided by upregulated genes may lead to the discovery of new treatment targets. However, more investigation, functional analysis, and validation of differentially expressed genes are essential to improve therapy choices and get a thorough grasp of AD's complexity.

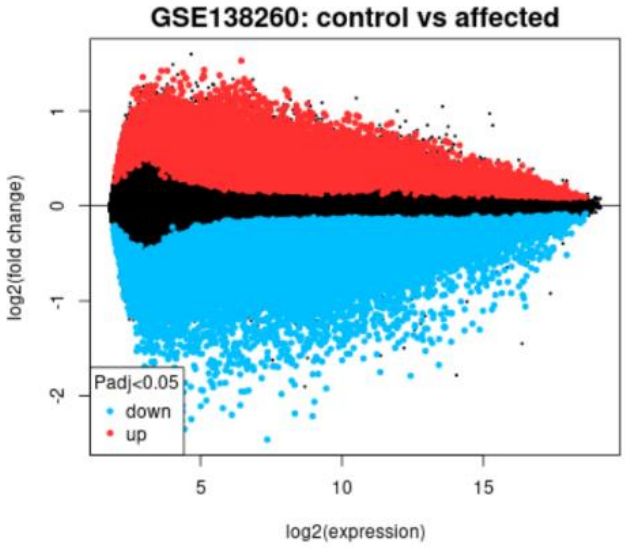

Fig 3: Mean- difference Plot

The differences in gene expression between people with and without Alzheimer's disease (AD) may be clearly shown in a mean difference plot. Positive numbers denote genes that are elevated in AD, whereas negative values denote genes that are downregulated in AD. Significant expression discrepancies, the effect of AD on gene expression, and putative underlying processes are all made clear by this graphical depiction. For the results to be reliably and accurately interpreted, statistical analysis is essential.

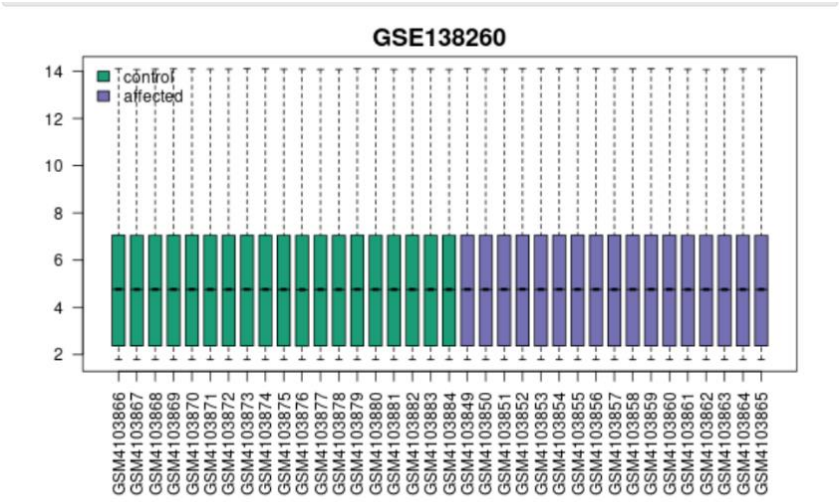

Fig 4: Box Plot

Box plots are used to assess the central tendency, dispersion, and variability of gene expression levels in healthy and Alzheimer's-affected individuals. Genes with consistent up- or down-regulation can be found, while outliers show unusual patterns of expression. The explanation of detected discrepancies is correct thanks to statistical analysis.

4.2 Molecular Docking

The outcomes of our molecular docking study, which looked at how phytochemicals from Capparis decidua interact with Acetylcholinesterase (AChE), show that natural biomolecules have the capacity to obstruct the enzyme by attaching to its active site residues. 18 of the 55 chosen phytochemicals showed binding affinities similar to the common inhibitor. The pharmacokinetic features of six phytochemical compounds with promising binding energies were further examined. Linolenic acid (-7.8 Kcal/mol) and 2-hydroxycinnamic acid (-7.1 Kcal/mol), which showed the lowest binding energies and the strongest affinity for AChE inhibition, distinguished themselves as the lead compounds.

Our study's two lead compounds showed binding affinities that were equivalent to those of FDA-approved cholinesterase inhibitors like Donepezil (-7.0 Kcal/mol). We used SwissTargetPrediction, an online application based on machine learning, to evaluate these findings. SwissTargetPrediction supported the potential of these phytochemicals in downregulating PARP-1 in Alzheimer's disease based on ongoing studies.

ADME profiling was done on phytochemicals to evaluate their pharmacokinetic characteristics. Table I lists substances with binding energies equivalent to those of a typical inhibitor and lists the many ways in which they interact with amino acid residues. Six of these compounds—Linolenic Acid, Linoleic Acid, Palmitic Acid, 2-hydroxycinnamic Acid, Myristic Acid, and Salicylic Acid—showed interactions with the amino acid residues of the important target protein AChE. The chemicals shown in Figures 3 and 4 showed that they could pass across the blood-brain barrier (BBB), which protects the brain from the circulation. SwissADME was used to evaluate the selected phytochemicals for ADME data analysis in order to further study the pharmacokinetic properties. The bioavailability score, Lipinski's Rule of Five (RO5) adherence, and BBB permeability of these favoured drugs were also evaluated. The findings showed that these phytochemicals have favourable characteristics that matched these criteria. The phytochemicals that effectively penetrated the BBB were noted in Table II as positive[58].

Table II. Phytochemicals with binding affinity and binding interaction residues

Ligand	**Binding Energy In (kcal/mol)**	**Interacting Amino Acids**
Isorhamnetin	-9.4	Hydrophobic Interactions: PHE 297, TYR 341 H bonds: TYR 72, PHE 295 pi-stacking: TRP 286, TYR 341
Capparisinine	-9.1	Hydrophobic Interactions: TYR 72, PRO 537, LEU 540, LEU 76, TRP 86, GLN 527, ALA 528, VAL 239, ARG 524, GLU 243, GLU 296, ARG 246, VAL 408 TRP 286, TYR 382, , LEU 289, VAL 330, TYR 337, LEU 524, PHE 338, TRP 532 , TYR 341, , VAL 429, , H bonds: TYR 72, GLY 120, TYR 124, ARG 525, SER 125, ASP 400, GLU 431, TYR 133, GLU 202, HIS 287, PHE 295, ALA 526, GLN 527, LYS 332, TYR 337, TYR 382, Salt bridges: LYS 332, ASP 400, ARG 525
Capparidisine	-8.9	Hydrophobic Interactions: GLN 527, ALA 528 H bonds: TYR 382, ASP 400, ALA 526, GLN 527
beta-Sitosterol-beta-D-glucoside	-8.3	Hydrophobic Interactions: TYR 382, GLN 527, GLN 527 H bonds: ARG 525
Linolenic acid	-7.8	Hydrophobic Interactions: TYR 72, TRP 86, PHE 338, TYR, TRP 286, PHE 297, TYR 337, 341 H bonds: TYR 124
beta-Sitosterol	-7.8	Hydrophobic Interactions: LEU 289, TYR 341, TYR 72, LEU 76, TRP 286, H bonds: HIS 287
beta-Carotene	-7.5	Hydrophobic Interactions: VAL 239, GLU 243, ARG 246, TRP 532, PRO 537, LEU 540
Stearic acid	-7.2	Hydrophobic Interactions: TRP 86, TRP 286, PHE 338, TYR 341
Oleic acid	-7.2	Hydrophobic Interactions: TYR 72, TYR 341, TRP 86, TRP 286, PHE 338, PHE 297, TYR 337 H bonds: TYR 124, SER 125
2-Hydroxycinnamic acid	-7.1	Hydrophobic Interactions: TRP 86, TYR 337 H bonds: TYR 341, TYR 337 Pi- stacking: TRP 86
Arachidic acid	-7	Hydrophobic Interactions: TRP 286, TRP 86, TYR 337, TYR 72, PHE 338, TYR 341 H bonds: TYR 124, SER 125
Donepezil	-7	Hydrophobic Interactions: GLU396A, ASP400A, TYR382A.
2-Hydroxy-6-methoxybenzoic acid	-6.9	Hydrophobic Interactions: VAL 330, VAL 408, VAL 429, LEU 524, ARG 525 H bonds: LYS 332, GLU 431, ARG 525 Salt Bridges: LYS 332, ARG 525
Salicylic acid	-6.6	H bonds: GLY 120, GLU 202 pi-stacking: TRP 86
3,4-Dihydroxybenzoic acid	-6.3	Hydrophobic Interactions: TYR 337 H bonds: GLY 120, TYR 124, GLU 202, TYR 337 Pi-stacking: TRP 86
Linoleic acid	-5.7	Hydrophobic Interactions: VAL 429, LEU 524, ARG 525, GLN 527
Myristic acid	-5.6	Hydrophobic Interactions: PHE 297, PHE 338, TYR 341, LEU 289, H bonds: SER 293
Palmitic acid	-4.8	Hydrophobic Interactions: PRO 410, TRP 532, LEU 536, PRO 235, PRO 537, VAL 370, LEU 540

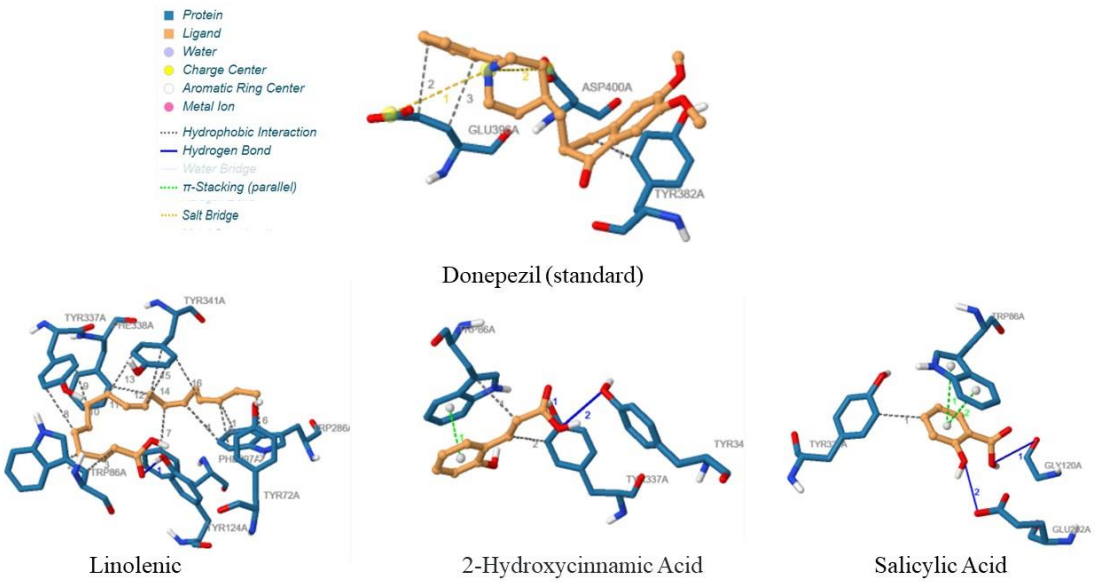

Figure 5: Structure and Interaction of Donepezil, Linolenic Acid, 2-hydroxycinnamic Acid with the target AChE.

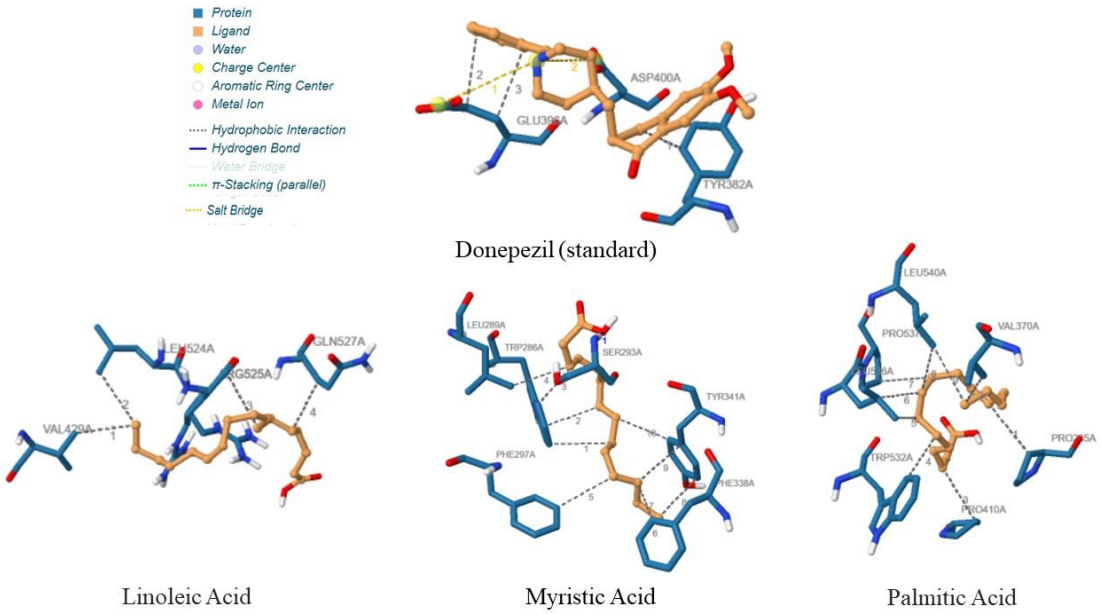

Figure 6: Structure and Interaction of Donepezil, Linoleic Acid, Myristic Acid Palmitic Acid with the target AChE.

According to Lipinski's ruleFor a substance to be deemed orally active, it must have a molecular mass below 500 Daltons, a molar refractivity between 40 and 130, a low logarithmic octanol/water partition coefficient (LogP), no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. If only one of these five requirements is broken by a chemical, it can still be appropriate for oral action.

The assessment of a few phytochemicals using Lipinski's Rule of Five (RO5) is shown in Table III. Salicylic acid, 2-hydroxycinnamic acid, and myristic acid all showed zero violations of RO5, showing that they have characteristics that meet the requirements for oral bioavailability. But just one violation was visible for linoleic acid, palmitic acid, and linolenic acid. Despite this slight departure, they continue to follow the overall standard of having just one violation, successfully passing the Lipinski criteria for each of the six compounds.

Table III: Analysis of Lipinsk's Rule of Five (RO5)

RO5 analysis	Linolenic acid	2-Hydroxycinnamic acid	Salicylic acid	Linoleic acid	Myristic acid	Palmitic acid
Molecular Mass	280	164	138	280	228	265
Hydrogen Bond Donor	1	2	2	1	1	1
Hydrogen bond Acceptor	2	3	3	2	2	2
LogP	5.8845	1.49	1.0904	5.8845	4.7721	5.5523
Molar Refractivity	86.993774	44.776596	35.066097	86.993774	68.713783	77.947777

Table IV displays the bioactivity rankings for the chosen phytochemicals as determined by the Molinspiration online application. These ratings represent how the phytochemicals interact as ligands with various receptors, such as nuclear receptors, GPCRs, enzymes, proteases, and kinases, as well as ion channel modulators. The precise pharmacological effects that the phytochemicals create are a result of these interactions.

Table IV: Bioactivity scores of six selected phytochemicals

Phytochemical	GPCR	PI	EI	NRL	KI	ICM
Linolenic Acid	0.33	0.13	0.42	0.35	-0.19	0.23
2-Hydroxycinnamic Acid	-0.64	-0.9	-0.21	-0.25	-0.97	-0.37
Salicylic Acid	-0.98	-1.14	-0.41	-0.79	-1.22	0.43
Linoleic Acid	0.29	0.12	0.38	0.31	-0.16	0.17
Myristic Acid	-0.11	-0.19	0.13	-0.06	-0.51	0.03
Palmitic Acid	0.02	-0.04	0.18	0.08	-0.33	0.06

Additionally, a second online tool is used to compute bioavailability scores, which show the proportion of substances anticipated to reach systemic circulation while taking into account aspects like absorption, metabolism, and excretion. Fig.7 lists possible compounds' chances of getting to their designated target sites and exerting therapeutic effects based on their bioactivity.

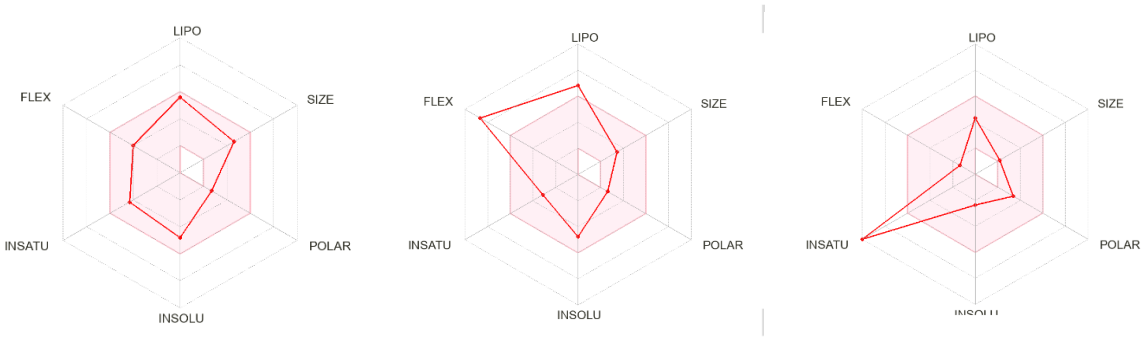

Figure 7: Bioavailability radars of Donepezil, Linolenic Acid and 2-Hydroxycinnamic Acid respectively retrieved using swisADME

Table II.: Blood Brain Barrier pearmeability of selected phytochemicals

Phytochemical	BBB +/-
Linolenic Acid	Positive
2-HydroxycinnamicAcid	Positive
Salicylic Acid	Positive
Linoleic Acid	Positive
Myristic Acid	Positive
Palmitic Acid	Positive

DISCUSSION

Plants have been used as a source of medicine for treating a variety of disorders for a very long time. They have enormous cultural and historic value, and their accessibility, affordability, and availability play a crucial part in the development of cutting-edge treatment modalities. Numerous secondary metabolites, including as flavonoids, phenolic compounds, alkaloids, and others, are present in these therapeutic plants. These bioactive substances, which plants produce as a kind of defence, frequently display exceptional medical qualities, increasing their therapeutic potential and medicinal worth.

Therapeutic research is significantly hampered by Alzheimer's, the major cause of dementia globally. By quickly hydrolyzing acetylcholine (ACh), acetylcholinesterase (AChE), a highly selective enzyme, contributes significantly to the reduction of cholinergic transmission. Butyrylcholinesterase (BChE) levels rise whereas AChE levels sharply decline in patients with advanced illness. This compensatory mechanism implies that preventing AChE activity might enhance cholinergic transmission by preventing ACh in the synaptic cleft from being broken down. By maintaining ACh levels, cholinesterase inhibitors show promise for improving cognitive performance and easing Alzheimer's symptoms. The creation of efficient treatment approaches depends on recognising and combating this enzymatic imbalance.

By using algorithms to find the target receptor's active region, such as AChE, and generate a binding pocket for ligands, molecular docking is a useful technique for identifying new inhibitors. Lower scores indicate higher affinity, while the docking score measures the binding

affinity between the receptor and ligands. Using this method, numerous chemicals may be tested for their potential to bind with the receptor and limit its function. Researchers may screen and prioritise candidate compounds that have promising binding affinity with the target receptor by using molecular docking, which speeds up the search for new inhibitors.

In this study, gene enrichment analysis was used to look into the underlying genes connected to the illness. A potent approach for locating certain genes or biological processes that are strongly enriched in a given dataset is gene enrichment analysis. We discovered the AChE gene's elevation by comparing the gene expression patterns of Alzheimer's disease (AD) patients and controls, as this gene is known to be crucial in the decrease of cholinergic transmission.

We used molecular docking using Autodock Vina to further investigate possible AChE inhibitors. A computer method called molecular docking is used to forecast the interaction and affinity between target proteins (receptors) and small molecules (ligands). In this instance, our attention was drawn to the phytochemicals produced by Capparis decidua, specifically Linolenic Acid and 2-hydroxycinnamic Acid.

These phytochemicals have better AChE binding affinities than the FDA-approved AChE inhibitor, Donepezil, according to the docking results. The affinity of binding of linolenic acid was -7.8 Kcal/mol, whereas that of 2-hydroxycinnamic acid was -7.1 Kcal/mol. Surprisingly, compared to donepezil, which exhibited a binding affinity of -7.0 Kcal/mol, both phytochemicals showed greater affinities towards AChE.

The phytochemicals from Capparis decidua may operate as potential AChE inhibitors, enhancing cholinergic transmission and maybe having therapeutic benefits in the treatment of Alzheimer's disease, according to these findings.

Investigating different therapeutic approaches increases the likelihood of overcoming Alzheimer's disease, which inspires hope for the creation of more effective therapeutic treatments. In order to provide a wide variety of alternatives for controlling AD, it is critical for research in this field to assess the efficacy and safety of these alternative therapies. It is crucial to support more research and encourage clinical trials in order to advance our understanding of the condition and discover viable treatments for it. By doing this, we can make substantial strides in our knowledge of Alzheimer's disease and find brand-new medicines that may enhance patient outcomes and quality of life.

CHAPTER 5: CONCLUSION

This study's main goal is to determine if certain phytochemicals obtained from Capparis decidua, specifically linolenic acid and 2-hydroxycinnamic acid, have any inhibitory effects on the AChE enzyme. The objective is to investigate their potential as Alzheimer's disease treatment options.

In order to cure Alzheimer's disease, this study emphasises the possible inhibitory effects of two phytochemicals, linolenic acid and 2-hydroxycinnamic acid, isolated from Capparis decidua. These compounds have qualities that are advantageous for drug development, according on assessments of their pharmacokinetic and drug-likeness features. To validate their therapeutic applicability, additional It is necessary to validate through in vivo and in vitro study.

The inadequacies of current Alzheimer's disease therapies draw attention to the need for development. The synthesis of neuroprotective medicines derived from medicinal plants is now a potential possibility because to the combination of genomic and metabolomics technology. It is now feasible to alter secondary metabolic pathways in plants, increasing the synthesis of useful substances, by using genome and transcriptome data.

Additionally, gene enrichment analysis was essential in identifying the dysfunctional gene AChE as a significant contributor to Alzheimer's disease. The ensuing molecular docking studies shed light on the chosen phytochemicals' AChE enzyme binding affinities. This information paves the way for possible medication development, together with assessments of drug similarity and pharmacokinetic profile.

This study intends to address the global demand for Alzheimer's disease treatments that are both economical and efficient and are derived from natural ingredients. It is important to recognise the fact that there is a study gap on the nutritional benefits of herbal medicine. Expanding our knowledge of the condition and strengthening our arsenal against its advancement require more investigation and evaluation of these qualities.

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Summary

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CERTIFICATE

I hereby certify that the Project Dissertation titled "Evaluation of *Capparis Decidua*'s potential as an alternative approach for AD: A gene enrichment and molecular docking study." which is submitted by Shipra Rathour, 2K21/MSCBIO/47, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 30/5/23

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