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Exploring Triphala's Therapeutic Potential: Targeting AD Pathology Proteins BACE1, GSK3β, and MMP9 through In- Silico Approaches

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ABSTRACT

Alzheimer's disease (AD) and associated dementia have emerged as prominent global health concerns over recent decades. Characterized by complex genetic, epigenetic, and metabolomic alterations, AD remains incurable. Extensive exploration into the genetic landscape of AD pathology has led to the identification of several biomarkers strongly implicated in the disease, including GSK3ß, BACE1, and MMP9, alongside the hallmark APP and Tau-related neurofibrillary tangle formation. While certain commercially available drugs such as memantine and donepezil are utilized in AD treatment, their efficacy is subject to debate, with notable side effects. Consequently, attention has turned to Ayurvedic herbal components, frequently consumed in India, renowned for their diverse health benefits and comparatively lower adverse effects. In this investigation, Triphala, a composite formulation comprising extracts from *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellerica*, was selected for the study. Through identification of the bioactive constituents of these plant extracts, molecular docking analyses were conducted with GSK3ß, BACE1, and MMP9 proteins. Findings indicate that the bioactive constituents of Triphala exhibit enhanced interactions with these target proteins, suggesting a potential improvement in AD pathology through Triphala consumption. However, it is imperative to underscore that further molecular experimentation in laboratory settings is essential to validate these initial findings conclusively. Looking ahead, the composition of Triphala holds promise as a prospective therapeutic agent for the treatment of AD-associated dementia.

Keywords: Alzheimer's Disease (AD), Triphala, Biomarkers, GSK3ß, BACE1, MMP9, Emblica officinalis, Terminalia chebula, Terminalia bellerica, APP, molecular docking.

INTRODUCTION:

The most prevalent type of dementia, Alzheimer's disease (AD), is primarily affecting senior people over 60. It is a gradual degenerative brain illness. Because of longer lifespans linked to better diet and healthcare, the prevalence of AD and dementia is rising quickly. According to estimates, millions of individuals worldwide suffer from dementia, and by 2050, that figure is predicted to increase to over 120 million. [1,2] For around eight years, there is a progression from the first moderate symptoms to severe dementia and finally death. It is recognized, therefore, that the illness has a protracted preclinical period

that lasts for over two decades. Once symptoms appear, a clinical diagnosis is usually made in routine basis.

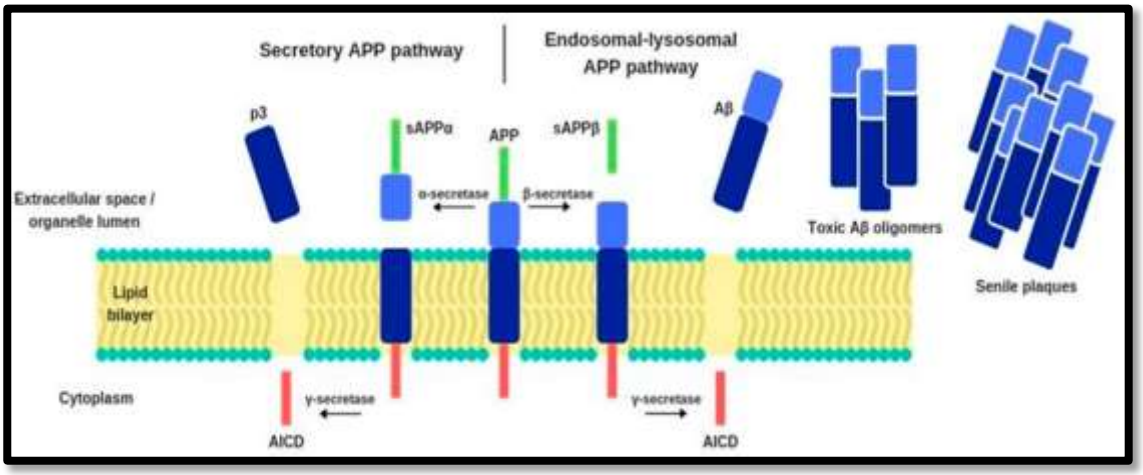


Fig 1: Biochemical pathways of Amyloid Precursor Protein (APP) modifications

AD-related behavioral abnormalities are a result of underlying CNS-related processes. The etiopathogenesis of this condition remains incompletely understood despite ongoing study. [3] On the other hand, several distinctive mechanisms have been found in both tissues and cells. Amyloid beta (Aβ) buildup is a common mechanism seen in AD. Aβ is a short peptide derived from naturally occurring amyloid precursor protein (APP) that accumulates to create senile plaques. Under physiological settings, Aβ contributes to axonal development, regulates key processes, and affects synaptic plasticity. (Fig 1) [4-6]

Under typical circumstances, the APP is broken down via the secretory pathway, which involves moving it from the endoplasmic reticulum to the Golgi apparatus and then to the cellular membrane, where it is subjected to proteolytic alterations. [7,8] α-secretase is the enzyme that catalyzes the process, cleaving APP into two fragments: a soluble sAPPα protein and another that γ-secretase further proteolyzes. An intracellular APP domain (AICD) and a p3 fragment are produced by this mechanism. A different endosomal-lysosomal APP proteolytic processing route may, nonetheless, be accessible for APP under diseased circumstances. The biochemical changes of APP are also influenced by β-secretase, which functions in the vicinity of the cellular organelles' lumen, adjacent to the APP N-terminus, whereas γ-secretase acts in the vicinity of the cytoplasmically submerged APP C-terminus. The β- secretase enzyme catalyzes the production of the sAPPβ protein. Along with AICD, another insoluble peptide produced by this mechanism is amyloid beta (Figure 1). The alternate APP cleavage by β- secretase is 50% more common in AD patients compared to healthy persons. As a result, the extracellular space contains a high concentration of Aβ, which binds to microglia, astrocytes stimulated by proinflammatory cytokines, degraded axons, and apolipoprotein E (APOE). [9,10] Senile plaques are able to pierce blood arteries, cutting off the brain's blood supply. In addition, they injure neurons and trigger astrocytes, microglia, and the complementing system to become active. These mechanisms are associated with increased death of neurons due to elevated generation of free radicals and increased Ca2+ ion influx. Moreover, Aβ can trigger the production of proinflammatory chemicals such prostaglandins, excitotoxins, and cytokines, including tumor necrosis factor α (TNF-α), as well as activate the receptors for advanced glycation end products on the surface of neurons. Inflammation as a result impedes brain

function and ultimately results in cellular death.

Other pathological alterations that mostly impact the structure of pyramidal neurons are frequently present in conjunction with Aβ production. Tau proteins undergo elevated phosphorylation, which results in the formation of tau tangles, which are polymers (Fig 2). Tau proteins are crucial for the stability of microtubules, the cytoskeleton's structural components, under physiological circumstances. In order for neurons to operate properly, especially for proper synaptic communication, microtubules involved in the movement of cellular proteins and enzymes.

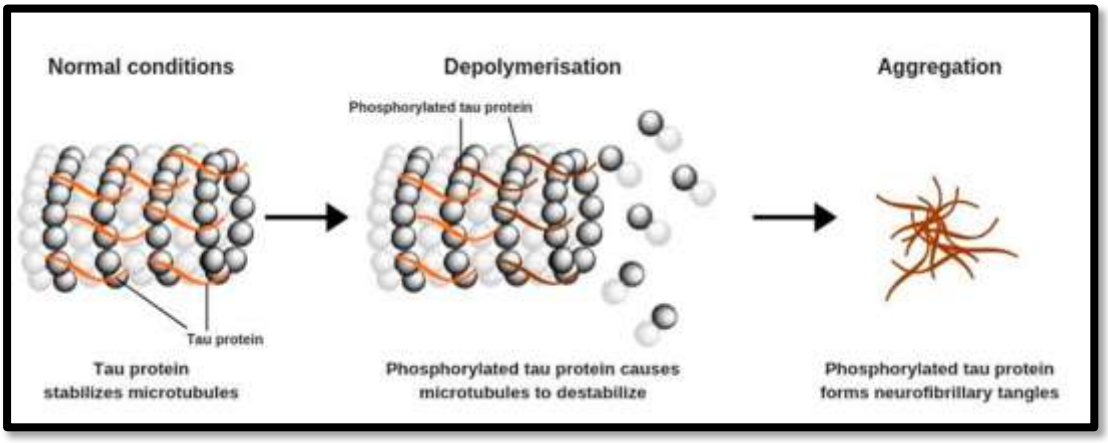


Fig 2: Hyperphosphorylation of Tau protein

Proper nutrition is a key component of a healthy lifestyle, and it seems to play a crucial role in the prevention of neurodegenerative diseases, including AD. A balanced diet rich in bioactive compounds can reduce the risk of dementia [11,12]. Given the scarcity of human interventional studies it remains unclear whether these substances also exert all of the neuroprotective effects observed in in vitro and animal model studies in human under physiological conditions. There is also a lack of knowledge as to whether the amounts and chemical forms present in food make them sufficiently bioavailable.

Herbal medicines appear to be a viable alternative to pharmaceuticals in treating illnesses because of their low cost, accessibility, and reduced potential for drug interactions [13]. This is likely owing to the excessive and indiscriminate use of medications, as well as their prices, sides, and interactions. The hunt for a novel medicinal plant-based drug to treat neurodegenerative diseases has therefore progressed significantly, and several research studies and documentation suggest that herbal remedies play a major part in the management of Alzheimer's disease. Given its abundance and diversity of phytoconstituents, including polyphenols, terpenes, anthocyanins, flavonoids, alkaloids, and glycosides, Terminalia chebula Retz. (Combretaceae) is one unique herbal treatment. The fruits of T. chebula are said to have been employed since ancient times in Unani, Ayurveda, and homeopathic medicine to cure geriatric illnesses and enhance memory and brain function. These fruits contain a variety of chemically active chemicals that give the plant its therapeutic properties [14,15]. Numerous ailments, including cancer, heart disease, paralysis, leprosy, ulcers, gout, arthritis, epilepsy, cough, fever, diarrhoea, gastroenteritis, skin conditions, urinary tract infections, and wound infections are also frequently treated with it.

However, the majority of the evidence for their beneficial benefits comes from experimental research elucidating the molecular processes behind the activity of potential bioactive substances in the prevention of AD, as well as observational epidemiological cohort studies. This review has documented

the prospective therapeutic effects of certain bioactive substances. Numerous of these chemicals fall into one of the following chemical classes: carotenoids, isothiocyanates, fat-soluble vitamins, and important omega-3 fatty acids.

1. *Emblica officinalis*:

Emblica officinalis genus Phyllanthus (Euphorbiaceae) also termed as *Phyllanthus emblica* or Indian gooseberry is widely distributed in tropical and subtropical parts of countries such as India, China, Indonesia (article amla pharmacology). *E. officinalis* commonly referred to as '*amla'* in India, is a small or moderate-sized tree with greenish-gray bark and greenish-yellow flowers which have been cited to possess vital amino acids and vitamins and therefore widely used as medicine and nutritious tonic. (Fig 2) [16] In comparison to other citrus fruits, it has a higher concentration of minerals and vitamin C. Furthermore, polyphenols, tannins, and epigallocatechin-3-gallat are among the chemical components of amla that have therapeutic effect [17]. For the treatment of several infectious and non-infectious disorders, all parts of E. officinalis, but especially the fruits, have been used as part of an Ayurvedic Rasayana, either by themselves or in conjunction with other traditional medicines. Many studies have demonstrated the potential benefits of *E. officinalis*, such as its ability to reduce inflammation, lower blood sugar, fight diabetes, lower cholesterol, fight cancer and its proliferative effects, stop oxidative stress, protect the heart and liver, and more. [18]

Fig : *Emblica officinalis* (Amla)

The remarkable effectiveness of Amla fruit extract in reducing heavy metal-induced toxicity, such as arsenic-induced oxidative damage and apoptosis in murine splenocytes, thymocytes, and hepatocytes, has also been demonstrated by a number of pre-clinical studies conducted by Singh et al. [19] Moreover, studies have shown that amla can effectively reduce immunotoxicity and inflammation brought on by arsenic exposure. [20]

2. *Terminalia chebula* :

Alzheimer's disease is a debilitating dementia, and only a limited number of therapeutic options are currently available to modify the manifestations of the disease. [21-28] *T. chebula* has pharmacological activities relevant to dementia therapy (Fig 3). Different extracts from *T. chebula* have exhibited concentration-dependent inhibitory

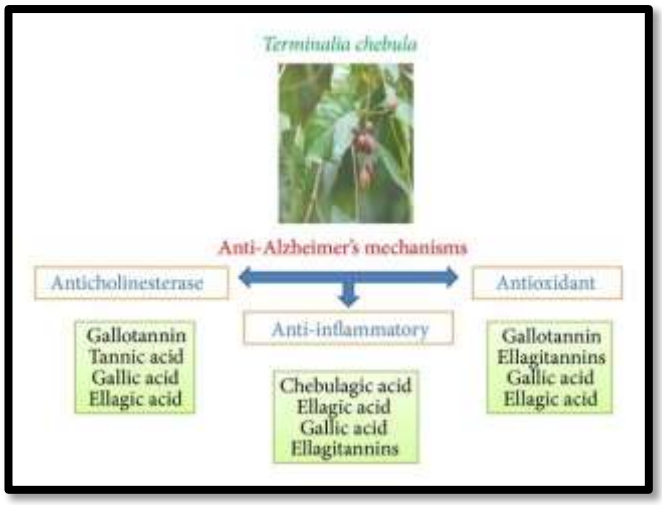


Fig 4: Anti-Alzheimer's mechanisms of *Terminalia chebula*

activities on AChE and BChE. Such AChE activities are also described for its active ingredients such as PGG, gallic acid, ellagic acid, and tannic acid. Anti-inflammatory properties of *T. chebula* have been well documented in different experimental systems that could be attributed to chebulagic or gallic

Fig 5: *Terminalia chebula*

acid. *T. chebula* with a high content of phenolic constituents exhibits strong antioxidant and neuroprotective properties in vitro and in vivo. The efficacy of *T. chebula* in treating AD should be compared with the current standard pharmacological treatment in animal and clinical testing and researches (Fig 4). Such studies should include the identification of the active principle(s) in order to improve the validation of the clinical trials. Until then, this review provides some evidences on the benefits of *T. chebula* in the treatment of Alzheimer's disease. [21]

3. *Terminalia bellerica* :

Terminalia bellirica (Gaertn.) Roxb., a member of the Combretaceae family, is indigenous to Bangladesh, Bhutan, Cambodia, China, Indonesia, Laos, Malaysia, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam, and another country. It is often linked with teak and found in monsoon forests, mixed deciduous forests, or dry deciduous dipterocarp woods. Named Bhibhitaki in Sanskrit, and locally named Behera in India, it is a huge deciduous tree that grows up to 900 meters in elevation over much of

India, with the exception of the parched western area [29]. In addition to playing a crucial role in the preparation of triphala, the fruits are frequently utilized either separately or in conjunction with other plant-based medications in a variety of

Fig 6: *Terminalia bellirica*

conventional therapeutic formulations within the native medical system. [30]. Numerous Nepalese ethnic groups have been known to eat the fruit of *T. bellirica* [31]. Ayurveda uses the fruits, which contain laxative, astringent, anthelmintic, and antipyretic qualities, to treat a variety of illnesses, including hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhea, coughs, and eye issues [32]. The fruits' various extracts have pharmacological qualities such as anti- inflammatory, antidiabetic, analgesic, and antidiarrheal [33]. The fruit's ethanolic extract has been shown to have hepatoprotective effects in addition to having excellent antioxidant qualities. The safe use of *T. bellirica* fruits is demonstrated by toxicity tests carried out in our lab [34,35]. The present investigation was conducted to assess the protective impact of the aqueous acetone extract of *T. bellirica* fruits, since it shown the highest level of antioxidant activity in our screening analysis (Fig 5) [36].

Aims:

1. Identification of Bioactive components of Plant Extracts of Emblica officinalis, Terminalia chebula and Terminalia bellerica.
2. Processing of Bioactive components of these plant extracts for neuroprotective purpose in Alzheimer's Disease.

Objectives:

- To screen and identify potential bioactive compounds from a diverse collection of phytochemicals in these plant extracts. Through a systematic approach, compounds will be selected based on their potential interactions with ß-secretase 1 (BACE1), Glycogen synthase kinase-3 beta (GSK3ß), Membrane metalloproteinase 9 (MMP9).
- To perform molecular docking studies to investigate the binding interactions between the selected bioactive compounds and the target proteins (BACE1, GSK3ß, MMP9).

- To analyse the results obtained from the molecular docking studies. This analysis will involve assessing the binding affinity, binding modes, and key interacting residues of the bioactive compounds BACE1, GSK3ß and MMP9.
- Comparison of effective response of Triphala with that of Allopathy medicines available in the market of Alzheimer's Disease.

Materials and Methods:

Methodology:

1. System Used:

The Windows 11, 64 bit Operating system was used for the study.

2. Software Used:

a. AutoDock Tools:

AutoDock Tools (ADT) is a molecular modeling software suite commonly used for docking small molecules into protein binding sites. It is a part of the AutoDock suite of programs developed by the Olson Laboratory at The Scripps Research Institute.(41) This tool is available as open source in: https://autodock.scripps.edu/ .

AutoDock Tools provides a user-friendly graphical interface for preparing ligands (small molecules) and receptors (usually proteins) for molecular docking simulations. It allows users to define the binding site, prepare ligand structures, set docking parameters, and analyze the results of docking experiments.

Some of the key features of AutoDock Tools include:

- Ligand Preparation: ADT enables users to build and edit ligand structures, assign atom types, generate 3D coordinates, and add charges and other necessary parameters.
- Receptor Preparation: Users can import protein structures, remove water molecules or other unwanted components, assign atomic types and charges, and prepare the receptor for docking experiments.
- Docking Simulations: ADT uses the AutoDock algorithm to perform molecular docking simulations, where ligands are docked into the receptor's binding site to predict their optimal binding conformations and binding energies.
- Visualization and Analysis: The software allows users to visualize the results of docking experiments, analyze binding poses, and evaluate binding energies to identify potential drug candidates or understand protein-ligand interactions.

  It's worth noting that AutoDock Tools is primarily used for academic research and drug discovery applications. It has been widely adopted in the scientific community and has contributed to numerous studies and publications in the field of molecular docking.

b. MGL Tools:

MGL Tools (Molecular Graphics Laboratory Tools) is a software package developed by the Molecular Graphics Laboratory at The Scripps Research Institute. It is designed to assist researchers in the visualization, analysis, and manipulation of molecular structures and related data.

MGL Tools provides a set of programs and libraries that can be used for various tasks in molecular modeling and computational chemistry.(42) This tool is available as open source in: https://ccsb.scripps.edu/mgltools/ .

Some of the key features and functionalities of MGL Tools include:

- Molecular Visualization: MGL Tools offers interactive 3D visualization capabilities, allowing users to display and manipulate molecular structures. It supports various file formats commonly used in molecular modeling, such as PDB, MOL2, and SDF.
- Molecular Editing: The software allows users to modify and edit molecular structures. This includes tasks like adding or deleting atoms, bonds, or residues, adjusting bond angles, and generating new structures.
- Structure Analysis: MGL Tools provides tools for analyzing molecular structures and properties. This includes measuring distances, angles, and dihedral angles, calculating molecular surface areas, and determining hydrogen bonding patterns.
- Energy Calculations: The software offers functionalities for performing energy calculations on molecular systems. This includes calculating potential energy, performing molecular mechanics simulations, and optimizing molecular geometries.
- Ligand and Protein Preparation: MGL Tools includes utilities for preparing ligand and protein structures for further computational analyses. This involves tasks such as adding hydrogen atoms, assigning charges, optimizing structures, and generating input files for molecular docking or molecular dynamics simulations.

MGL Tools is often used in conjunction with other software packages in the field of computational chemistry and molecular modeling, such as AutoDock (for molecular docking) and AutoDock Vina (an improved version of AutoDock). It provides a user-friendly interface and a range of tools to aid researchers in various aspects of their molecular modeling workflows.

c. PyRx:

PyRx is a software tool that is used for virtual screening and molecular docking studies. It provides a graphical user interface (GUI) for the AutoDock suite of programs, which includes AutoDock Vina and AutoDock 4. PyRx allows users to perform docking simulations and analyze the results to predict the binding affinity and orientation of small molecules with target proteins.(43) This tool is available as open source in: https://pyrx.sourceforge.io/ .

Here are some key features and functionalities of PyRx:

- Molecular Docking: PyRx utilizes AutoDock Vina and AutoDock 4 algorithms for molecular docking studies. It allows users to dock ligands into target protein binding sites to predict their binding poses and estimate binding affinities.
- Virtual Screening: PyRx enables high-throughput virtual screening of large compound libraries against target proteins. It helps researchers identify potential lead compounds or drug candidates by ranking and prioritizing compounds based on their predicted binding affinity.
- Graphical User Interface: PyRx provides a user-friendly GUI that simplifies the preparation of protein and ligand structures, configuration of docking parameters, and analysis of docking results. It allows users to visualize and interact with the docking results in a 3D environment.
- Compound Library Management: PyRx includes features for managing compound libraries. Users can import or create libraries of small molecules, set up virtual screening experiments, and analyze the screening results.
- Analysis and Visualization: PyRx offers various tools for analyzing and visualizing docking results. It allows users to view the 3D structures of protein-ligand complexes, analyze binding affinities, calculate binding energy scores, and generate reports and plots.

PyRx is open-source software and is available for Windows, macOS, and Linux platforms. It aims to provide an accessible and user-friendly platform for researchers and scientists to perform molecular docking and virtual screening studies.

d. Vina:

Vina is a molecular docking program and an improved version of the AutoDock software developed by Dr. Oleg Trott. It is designed to predict the binding modes and binding affinities of small molecules (ligands) with a target protein or receptor.(41) This tool is available as open source in: https://vina.scripps.edu/ .

Here are some key features and aspects of Vina:

- Molecular Docking: Vina uses a scoring function and an efficient optimization algorithm to perform molecular docking simulations. It explores the conformational space of ligands and predicts their optimal binding poses within the protein's binding site.
- Binding Affinity Prediction: Vina estimates the binding affinities between ligands and proteins by considering various energetic terms, including van der Waals interactions, electrostatic interactions, and desolvation energy. The scoring function is designed to prioritize ligands with more favorable binding energies.
- Efficiency and Speed: Vina employs a simplified representation of the protein's target site, which helps accelerate the docking process compared to AutoDock. It also uses multi-threading and parallel computing techniques to further enhance its computational efficiency.
- User-Friendly Interface: Vina can be accessed and utilized through various graphical user interfaces (GUIs) and software tools, such as PyRx. These interfaces provide an intuitive way to set up docking experiments, visualize the results, and analyze the docking poses and scores.
- Flexibility: Vina allows flexibility in terms of the ligand and protein preparation. It can handle ligands in various formats (e.g., PDB, MOL2) and supports the inclusion of flexibility in the protein structure, where certain side chains or regions can be allowed to move during docking.

  Vina has gained popularity due to its improved speed, efficiency, and accuracy. It has been widely used in computational drug discovery, virtual screening, and structure-based drug design projects.

e. PyMOL:

PyMOL is a powerful molecular visualization software widely used in the field of structural biology. It allows researchers to visualize and analyze three-dimensional (3D) molecular structures, such as proteins, nucleic acids, small molecules, and complexes.(44) This tool is available as open source in: https://pymol.org/2/ .

Here are some key features and functionalities of PyMOL:

- Molecular Visualization: PyMOL provides high-quality rendering of molecular structures, allowing users to view and manipulate them in 3D. It offers various representations, including ball-and-stick, space-filling, surface, cartoon, and ribbon representations, which help in understanding the structural details and relationships.
- Molecular Editing: PyMOL allows users to modify and edit molecular structures. They can perform tasks such as adding or deleting atoms, residues, or bonds, changing atom properties, adjusting the conformation, and optimizing the structure.
- Molecular Dynamics: PyMOL supports the visualization of molecular dynamics simulations. Users can load trajectory files from molecular dynamics simulations and animate the structures to observe

the dynamic behavior of biomolecules over time.

- Measurements and Analysis: PyMOL provides tools for measuring distances, angles, dihedral angles, and surface areas. It also offers functionalities for hydrogen bonding analysis, cavity identification, and electrostatic potential calculation.
- Image and Movie Generation: PyMOL enables the creation of publication-quality images and movies of molecular structures. Users can customize the rendering style, lighting, and colors, and export the images or movies in various file formats.
- Scripting and Integration: PyMOL has a powerful Python scripting interface that allows users to automate tasks, create custom functions, and build complex workflows. It also supports integration with other software and libraries for data analysis and molecular modeling.

PyMOL is available as both open-source and commercial versions. The open-source version provides basic functionalities, while the commercial version, PyMOL Enterprise, offers additional features, support, and maintenance.

f. Biovia Discovery Studio:

Biovia Discovery Studio, formerly known as Accelrys Discovery Studio, is a comprehensive software suite for computational drug discovery and molecular modeling. It is developed by Dassault Systèmes BIOVIA, a leading provider of scientific software solutions.

Discovery Studio offers a wide range of tools and capabilities to support drug discovery research, protein modeling, ligand design, virtual screening, and other molecular modeling tasks. This tool is available as open source in: https://discover.3ds.com/discovery-studio- visualizer-download .(45)

Here are some key features and functionalities of Biovia Discovery Studio:

- Protein Structure Modeling: Discovery Studio provides tools for homology modeling, protein structure prediction, and refinement. It enables researchers to generate 3D models of proteins based on sequence information and compare them to experimental structures.
- Ligand Design and Optimization: The software offers tools for ligand design and optimization, including virtual screening, de novo design, and fragment-based design. It allows users to generate and refine ligand structures, predict their binding affinity, and explore chemical space for lead identification.
- Molecular Docking: Discovery Studio includes molecular docking algorithms for predicting the binding poses and binding affinities of ligands with protein targets. It enables researchers to perform docking simulations, analyze and rank the docking results, and evaluate the protein-ligand interactions.
- Pharmacophore Modeling: The software supports pharmacophore modeling, which involves identifying the essential features and spatial arrangement required for ligand binding. It allows users to generate and validate pharmacophore models based on known ligand-protein interactions.
- Cheminformatics and ADME-Tox: Discovery Studio provides a suite of cheminformatics tools for analyzing chemical structures, calculating molecular properties, and predicting ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. These features assist in the evaluation and optimization of drug-like properties.
- Workflow Automation: Discovery Studio allows users to automate and streamline their computational workflows using a visual interface. It offers a graphical workflow builder to create complex protocols involving multiple computational steps.

- Integration with Other Tools: Discovery Studio integrates with various databases, external tools, and scientific software packages to enhance its capabilities. It facilitates data exchange and interoperability with other software used in the drug discovery process.

  Biovia Discovery Studio is widely used in the pharmaceutical industry, academia, and research institutions for drug discovery, lead optimization, and molecular modeling studies. It provides a comprehensive suite of tools and a user-friendly interface to support scientists in their computational drug discovery efforts.

3. Website Used:

a. RCSB-PDB:

The RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) website (https://www.rcsb.org/) is the primary online resource for accessing and exploring the wealth of information contained in the Protein Data Bank (PDB)(46). It serves as a portal to the world's largest collection of experimentally determined 3D structures of biological macromolecules.

Here are some key aspects and features of the RCSB PDB website:

- Search and Browse: The website provides powerful search capabilities, allowing users to search for structures based on various criteria such as keywords, authors, protein names, organisms, and experimental methods. Users can also browse through different categories, such as proteins, nucleic acids, complexes, and ligands.
- Structure Visualization: The RCSB PDB website offers interactive visualization tools that allow users to view and analyze 3D structures directly in the web browser. Users can explore the structures in various representations, zoom in and out, rotate, and interact with different components of the structures.
- Structure Summary and Details: Each structure entry in the RCSB PDB database is accompanied by a detailed summary page. This page provides essential information about the structure, including the authors, experimental method, resolution (if applicable), ligands, and related publications. It also includes links to related structures and sequence information.
- Download and Access: The website provides options to download the 3D structure files in various formats, such as PDB, PDBx/mmCIF, and FASTA. Users can access the raw data and metadata associated with the structures, including atomic coordinates, experimental data, and annotations.
- Data Analysis and Tools: The RCSB PDB website offers a range of tools and resources for analyzing and exploring the data. This includes tools for superimposing and aligning structures, calculating structural similarities, exploring ligand binding sites, and visualizing protein-ligand interactions.
- Educational Resources: The website includes educational materials and resources aimed at students, educators, and the general public. These resources provide an introduction to structural biology, tutorials on using the RCSB PDB resources, and interactive activities to learn about protein structures.
- Integration with External Resources: The RCSB PDB website integrates with various external databases and resources, such as UniProt, Gene Ontology, and PubMed. This integration enables users to access additional information and context related to the structures.

The RCSB PDB website is freely accessible to users worldwide and provides a user-friendly interface for navigating, searching, and exploring the vast collection of structural data available in the PDB. It

serves as a valuable resource for researchers, scientists, educators, and anyone interested in the field of structural biology.

b. PubChem:

The PubChem website (https://pubchem.ncbi.nlm.nih.gov/) is a comprehensive resource provided by the National Center for Biotechnology Information (NCBI), which is part of the
U.S. National Library of Medicine. PubChem is a free database that provides information on the biological activities, chemical structures, and properties of small molecules.(47)

Here are some key aspects and features of the PubChem website:

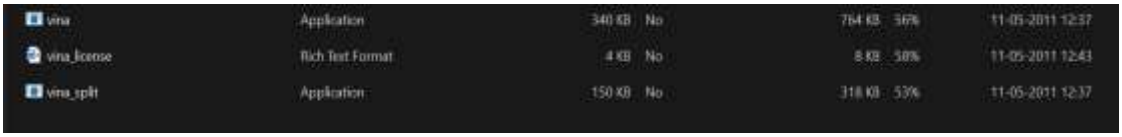
- Compound Database: PubChem maintains a vast collection of chemical compounds, including small organic molecules, peptides, and natural products. It provides detailed information on each compound, including its chemical structure, physical properties, synonyms, and links to relevant literature.
- Compound Search: The PubChem website offers a powerful search interface that allows users to search for compounds based on various criteria. Users can search by compound name, molecular formula, molecular weight, CAS registry number, or other identifiers. Advanced search options and filters enable more specific queries.
- Compound Summaries: Each compound in PubChem has a dedicated summary page that provides comprehensive information about the compound. This includes its chemical structure, IUPAC name, molecular weight, biological activities (if available), safety data, and links to related resources.
- Biological Activities: PubChem integrates information on the biological activities of compounds, including their interactions with biological targets, such as proteins, enzymes, and receptors. It aggregates data from various sources, including high- throughput screening assays, and provides access to bioactivity profiles and dose- response curves.
- Chemical Safety: The PubChem website includes information on the safety and hazards associated with chemical compounds. It provides data on chemical toxicity, environmental effects, and physical properties, helping researchers assess the safety and risks associated with specific compounds.
- Data Download and APIs: PubChem allows users to download compound data and bioactivity information in various formats, including SDF, SMILES, and XML. It also provides application programming interfaces (APIs) for programmatic access to the data, enabling integration with other software and databases.
- Tools and Resources: The PubChem website offers a range of tools and resources to aid chemical research and analysis. This includes tools for chemical structure searching, similarity searching, substructure searching, and compound clustering.

PubChem is widely used by researchers, scientists, and educators in various fields, including drug discovery, chemical biology, and toxicology. The website serves as a central hub for accessing chemical information and supports the exploration and analysis of small molecules.

Method:

1) AutoDock vina from the Centre for computational structural biology website (https://vina.scripps.edu/) was downloaded.

2) The autodock.msi program file was downloaded and installed.
3) The path of the installation was located. It should be C:\Program Files (x86)\The Scripps Research Institute\Vina .
4) A folder named 'Docking' was created and placed in desktop. Then The contents of the 'Vina' folder (Vina, Vina_license, Vina_split) were copied and pasted within the 'Docking' folder.

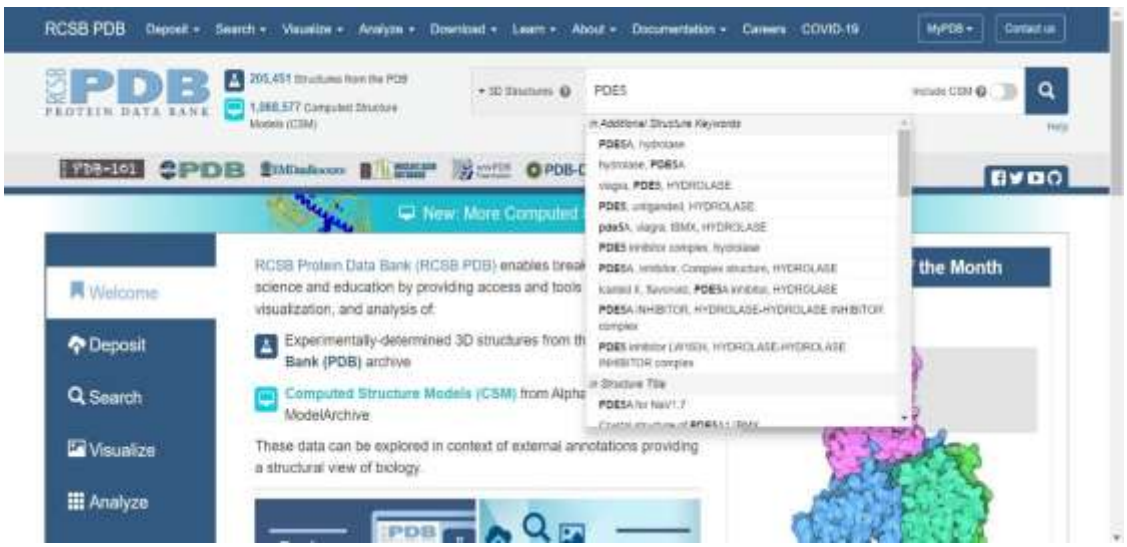


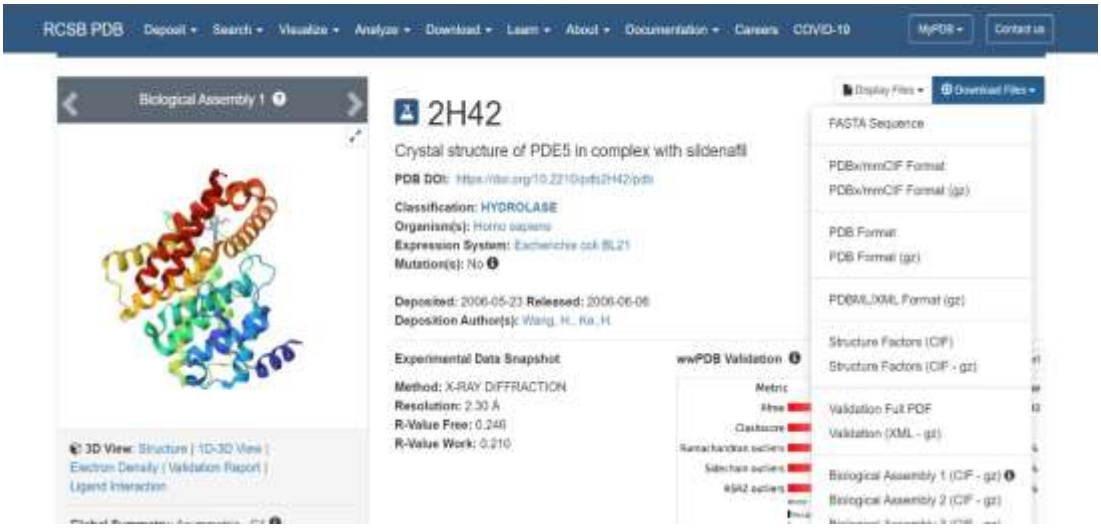
5) MGLtools from https://ccsb.scripps.edu/mgltools/downloads/ was downloaded and installed.

6) PyMol unlicensed version (https://pymol.org/2/) or Biovia Discovery Studio (versions from 2016-2021)(https://discover.3ds.com/discovery-studio-visualizer-download) were downloaded and

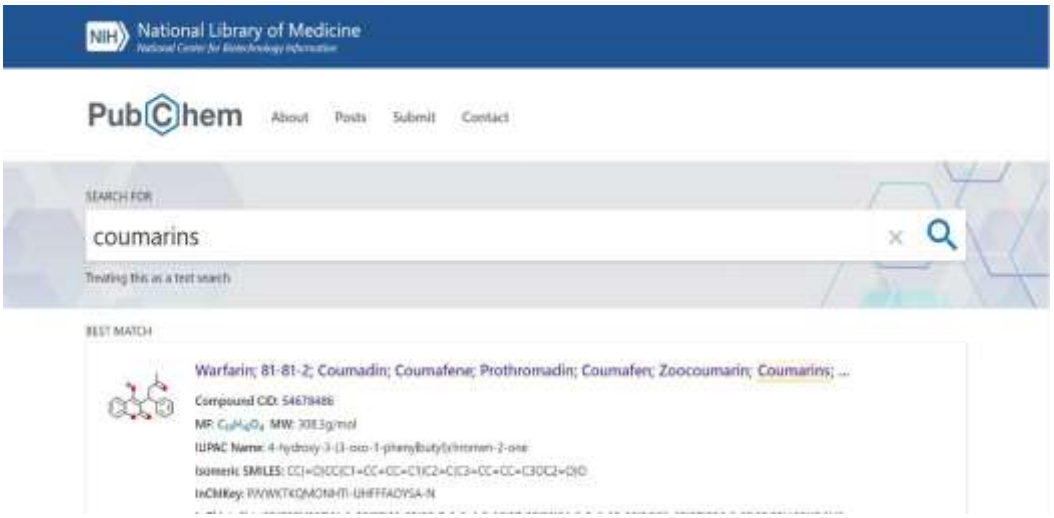
installed . These applications are needed for visualization of proteins and ligands.

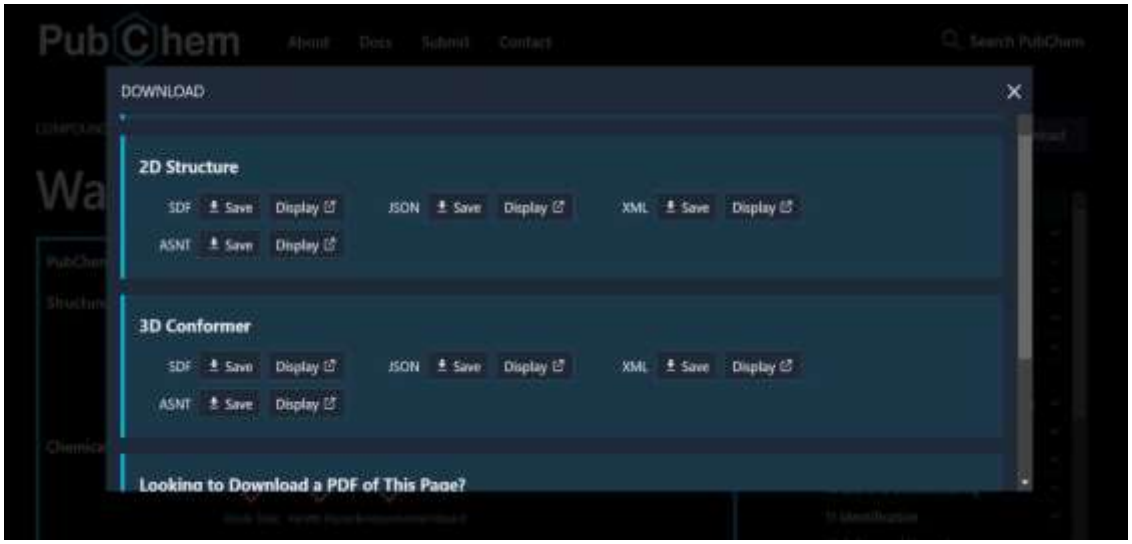
7) Thereafter, RCSB protein data bank (https://www.rcsb.org/) was accessed and our choice of proteins were downloaded in PDB format.





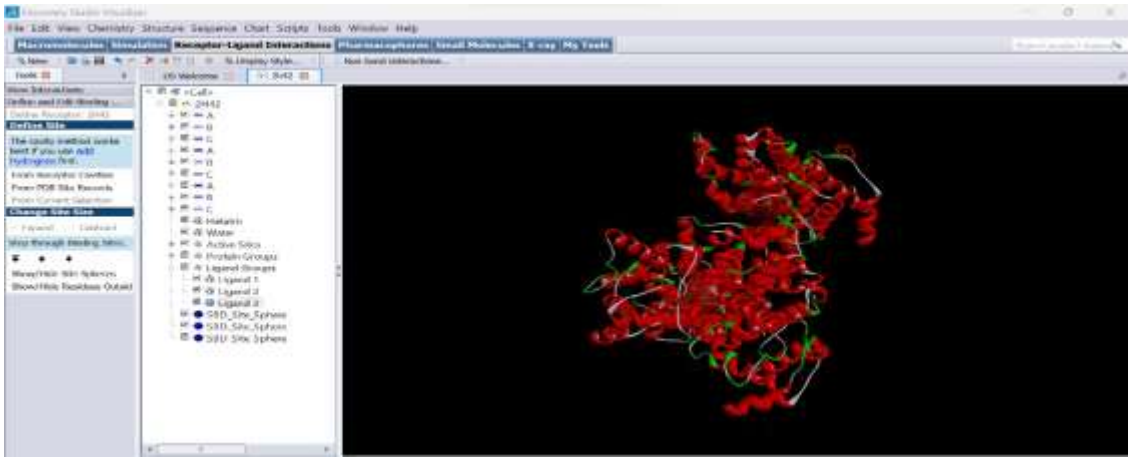
8) Our choice of ligands were downloaded from PUBCHEM (https://pubchem.ncbi.nlm.nih.gov/) and the 3D conformers were downloaded in .sdf format.





9) After that, all the downloaded files are copied and pasted to 'Docking' folder.
10) The ligand file Via Pymol/DiscoveryStudio/OpenbabbleGUI was opened and saved as .pdb format within the 'Docking' folder. Changed the name of the file to 'ligand'.

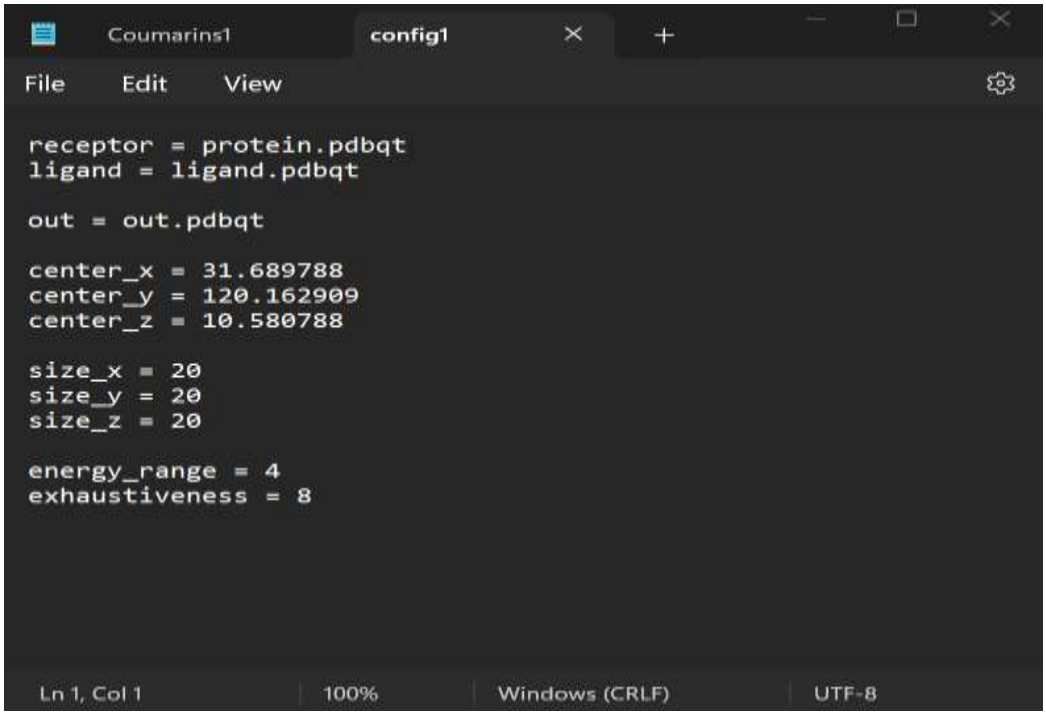
11) The protein file was opened via Pymol/Discovery Studio. The Water molecules and HetAtoms were deleted and polar hydrogen was added to the protein. After selecting the ligand groups, "Define and edit binding site" option was clicked to find "SBD_binding_sphrere". After right clicking on it we got the attributes that contains 3D XYZ coordinates of the site where the ligand was bound. This step is necessary for targeted docking. Saved the modified file in .pdb format within the 'Docking' folder. The file name was changed to 'protein'.



12) The Protein molecule was loaded in AutoDock Tool. To convert the protein from PDB format to PDBQT format, clicked on 'Edit' section. From 'charges' section, Kollman charges and compute Gasteiger Charges were added and assigned AD4 types from the 'Atoms section'. The modified file was saved as .pdbqt format with the same name 'protein'.
13) The ligand was loaded in AutoDock Tool and also saved it in .pdbqt format with the same name 'ligand'.
14) Under the Grid section, clicked on 'macromolecules' and thereby protein was chosen.
15) Under the Grid section, clicked on set map types and 'choose ligand'.
16) A config.txt file was created within the 'Docking' folder. The text content should look like this:

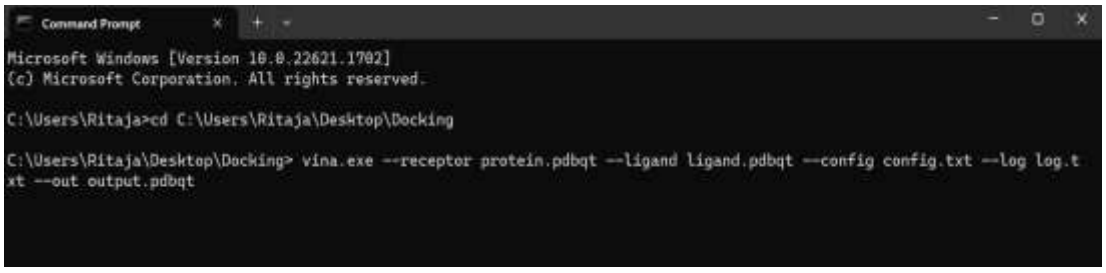
a. receptor = protein.pdbqt
b. ligand = ligand.pdbqt
c. out = out.pdbqt
d. center_x = -14.792429
e. center_y = 46.108089
f. center_z = -40.410036
g. size_x = 20
h. size_y = 20
i. size_z = 20
j. energy_range = 4
k. exhaustiveness = 8

(Note: The appropriate XYZ co-ordinates were found for targeted docking. Before protein preparation, the active site of it was found for the ligand interaction. In Biovia discovery Studio, the SBD site sphere was applied and located the target region. Attribute values were copied and pasted it in the config.txt file. Go to 'Grid box' section of the AutoDock tools and modified the XYZ wheels.)



17) 'Command Prompt' was opened. Entered the Docking folder following the 'cd' command. For example, if the 'Docking' folder is located within the 'Downloads' folder, the command will look like this: cd[space]C:\Users\'USERNAME'\Downloads\Docking.
18) Run this command:

vina.exe --receptor protein.pdbqt --ligand ligand.pdbqt --config config.txt --log log.txt -- out output.pdbqt



19) Best RMSD value (0.00) will rank at No.1 position, showing highest affinity. Log file contains the details of the docking and output.pdbqt file contains the protein docked with ligand. Used the data for further uses.

```
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -652418812
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -8.6      0.000      0.000
   2         -8.0      0.841      2.416
   3         -7.8      3.108      6.217
   4         -7.8      1.576      4.100
   5         -7.7      2.233      3.211
   6         -7.7      1.483      4.169
   7         -7.6      2.274      5.904
   8         -7.6      2.262      5.527
   9         -7.4      2.612      4.871
Writing output ... done.
```

Ln 1, Col 1 | 100% | Windows (CRLF) | UTF-8

20) Run this command:

vina_split.exe --input output.pdbqt

This will split the output.pdbqt file into separate files. All the files were imported into Biovia Discovery Studio for further analysis.

RESULT:

Characterisation of the ligands:

In my study, I have selected various phytochemicals and synthetic components which are characterized to have potential biological impact on neurodegenerative disoders. We have selected three medicinal plant extracts namely Emblica officinalis (Amla), Terminalia chebula (Chebulic Myrobalan) and Terminalia bellirica (Belleric Myrobalan) and studied their molecular structure. We have also determined their LC50 value. After initial screening, we have shortlisted from our initial selection of 30 ligands to 13 ligands on basis of its neuroprotective properties. These 13 ligands have shown significant association (on the basis of their docking score) with the target proteins of our study i.e., BACE1, GSK3ß and MMP9. The docking scores of these ligands are obtained by considering the highest affinity i.e., free energy value ΔG (Kcal/mol) with lowest RMSD (0.000).

All the proteins have different ligand binding site by default and developed evolutionarily. BACE1, GSK3ß and MMP9 have ligand binding site of 1, 5 and 7 respectively. We have identified their ligand binding site by targeting their associated ligands (For BACE1, the established PDE5 inhibitor is Chebulinic acid/Ellagic acid; For GSK3ß, the ligands are Corilagin , Rutin and Catechol and for MMP9, the ligands are Ellagitannins and Galloylpunicolagen). XYZ coordinates for these ligands are listed in table 1.

Proteins	3D Co-ordinators		
	X	Y	Z
BACE1 Site	22.074140	-24.810351	-0.129470
GSK3ß Site	124.366049	152.698077	137.155923
MMP9 Site1	3.233700	8.326300	22.094325
MMP9 Site2	0.043825	-1.633425	29.136925

Table 1: Different ligand binding sites of Bioactive components of Plant extracts are represented with their 3D coordinates

After that we have taken into account the various allopathy medicines which are available in the market against these proteins.

The medicines for BACE1 selected after screening are :

1. Verubecestat (MK-8931)
2. Lanabecestat (AZD3293)
3. Atabecestat (JNJ-54861911)
4. Elenbecestat (E2609)
5. CNP520

The medicines for GSK3ß selected after screening are:

1. Benzofuran-3-yl-(indol-3-yl)
2. 9-ING-41
3. SB-732881-H
4. AR-A014418
5. BIO
6. CHIR99021
7. AZD-1080
8. Lithium
9. Metavert

The medicines for MMP9 selected after screening are:

1. Batimastat
2. Marimastat
3. CGS-27023A
4. CGS-25966
5. Tanomastat
6. Prinomastat

We have also docked these ligands to their respective protein to determine the cut off threshold value to consider whether the phytochemicals/synthetic compounds have better efficiency to interact with the proteins or not. Docking scores for each ligand were listed below for the proteins.

BACE1

SL NO.	**LIGANDS (PLANT EXTRACTS)**	**AFFINITY (kcal/mol)**	**LIGANDS (MEDICINE)**	**AFFINITY (kcal/mol)**
1	Ascorbate	**-9.0**	CNP 520	-7.2
2	Catechol	**-8.9**	Lanabecestat	-6.9

3	Chebulagic Acid	-	Atabecestat	-6.9
4	Chebulinuc Acid	**-9.1**	Elenbecestat	-7.2
5	Corilagin	**-9.0**	Verubecestat	-7.2
6	Ellagitannins	**-9.0**		
7	Gallic Acid	**-9.0**		
8	Galloylpunicolagen	**-9.0**		
9	Quercetin	**-9.0**		
10	Rutin	**-9.0**		
11	Tannic Acid	**-9.0**		
12	Triterpinoids	**-9.0**		

Table 2: Comparative Docking scores of different ligands of plant extracts with respective Medicines of protein BACE1. All the docking scores are calculated using Autodock Vina.

GSK3ß

SL.NO.	LIGANDS (Plant extracts)	AFFINITY (kcal/mol)	LIGANDS (MEDICINE)	AFFINITY (kcal/mol)
1	Ellagic Acid	**-8.6**	nzofuran-3-yl- (indol-3-yi) maleimides	-6.7
2	Tannic Acid	**-8.4**	9-ING-41	-8.1
3	Rutin	-4.2	SB-732881-H	-7.3
4	Corilagin	**-7.9**	AR-A014418	-6.0
5	Gallic Acid	-5.1	CHIR 99021	-6.5
6	Triterpinoids	**-8.6**	AZD-1080	-7.1
7	Catechol	-4.3	Metavert	-7.6

Table 3: Comparative Docking scores of different ligands of Plant extracts with their respective medicines of protein GSK3ß. All the docking scores are calculated using Autodock Vina.

MMP9

SL. NO.	LIGANDS (Plant Extracts)	Ligand 1 AFFINITY (kcal/mol)	Ligand 7 AFFINITY (kcal/mol)	LIGANDS (MEDICINE)	LIGAND 1 AFFINITY (kcal/mol)	LIGAND 2 AFFINITY (kcal/mol)
1	Chebulagic Acid	**-9.1**	**-9.0**	Batimastat	-6.2	-6.2
2	Ellagic Acid	**-8.9**	**-9.0**	Marimastat	-5.2	-5.6
3	Gallic Acid	**-9.0**	**-9.0**	Tanomastat	-6.5	-6.2
4	Rutin	**-9.0**	**-9.0**	Prinomastat	-6.5	-6.5
5	Quercetin	-4.3	-4.2			
6	Ascorbate	**-7.9**	**-8.6**			
7	Triterpinoids	-4.3	-4.2			
8	Catechol	-9.0	-9.0			

Table 4: Comparative docking scores of different ligands of plant extracts with their respective medicines of protein MMP9. All the docking scores are calculated using Autodock Vina.

REVIEW OF LITERATURE

The number of Americans with Alzheimer's disease is estimated to be 5.8 million. By 2050, there might be 13.8 million persons worldwide estimated to have Alzheimer's or other dementias. An estimated USD 290 billion was spent on healthcare costs and missed income for individuals with AD and those who care for them in the United States alone in 2019. It is projected that USD 1.1 trillion will be spent on AD in the US by 2050 [45,46]. The development of novel therapies for the avoidance and management of AD is thus critically needed. Although several novel methods for treating AD symptoms and developing disease-modifying therapies are presently being developed, it is unclear when these techniques will be released into the market [45-48]. a substantial change from a one-way therapy comprehensive, individualized, multi-therapeutic approach, especially for chronic and complex diseases such as AD, may be effective [49,50].

For the purpose of treating AD-associated cognitive loss, we have created and put into practice a thorough precision medicine strategy, and we have shown long-term improvement in 100 patients [51]. The tailored strategy focuses on metabolic indicators that are not at their peak. It has been shown that improving these metabolic parameters helps a number of people with MCI, SCI, early AD, or pre-AD disorders.

Medicinal herbs, with their many physiological activities that eventually improve memory and restore normal cognitive functioning, are one of the strategies we utilize to balance the metabolic parameters. More than 100 new medications are in the clinical development stage thanks to medicinal plants, which have been the most fruitful source of leads for drug development [52]. The most typical ways that herbs are given are as an extract, in isolation, or as a combination of multiple plants, like triphala. More effectiveness, less non-specific toxicity, and—above all—avoidance of medication resistance are possible when a single herb or a combination of herbs is administered. The lengthy history of safety and effectiveness of herbs is probably because to their many constituents and to the interactions of these different components with multiple physiological targets in the body [53-55]. Several pharmacological actions have established the synergy between the various elements in the plant [56]. Among the many complimentary methods of action that make up synergistic interactions include the reversal of resistance, immunomodulation, and reduction of side effects. Consequently, it is not unexpected that a single herb or a combination of plants is favoured over a solitary chemical or its derivative. [53,54,56,57].

The data shown here emphasizes the possibility of herbal remedies—which has not yet received much attention—and the adoption of comparable customized, systems-based treatment techniques for AD. Large-scale multicentric clinical studies and more research into the biological processes of the herbs are required to confirm the effectiveness of both the single herbs and the combined formulations in the treatment of Alzheimer's disease and cognitive impairment prior to the onset of the illness. Further thorough investigation is required to surmount methodological constraints, such as inadequate study design, comparatively small sample numbers, subpar outcome measures, and incorrect end-point choices [56,57]. Combinatorial sciences, high-throughput screening methods, and the historical body of knowledge underlying traditional medical systems are expected to facilitate the use of herbal products and formulations in the drug development process, thereby yielding new functional leads for AD.

RESEARCH GAP

The research gap addressed in this study pertains to the exploration of alternative therapeutic avenues for Alzheimer's disease (AD) and associated dementia. Despite the existence of commercially available

drugs like memantine and donepezil, their efficacy remains uncertain, and adverse effects are a concern. This prompts the search for alternative treatment options with potentially lower side effects. The study aims to bridge this gap by investigating the potential therapeutic benefits of Triphala, a traditional Ayurvedic formulation widely consumed in India for various health benefits. While Triphala's constituents have been individually studied for their pharmacological properties, its collective efficacy against AD pathology remains largely unexplored.

Consequently, the study seeks to fill this gap by conducting further molecular wet lab experimentation to corroborate the initial computational findings and provide a more robust understanding of Triphala's efficacy in mitigating AD pathology. By addressing this research gap, the study aims to contribute to the development of novel therapeutic strategies for AD that leverage the benefits of traditional herbal medicine while minimizing potential side effects.

CONCLUSION:

From my above research we can conclude from the result that the bioactive components of Triphala are more effective and beneficial for its role in neuroprotective purposes against the neurodegenerative behaviour of Alzheimer's Disease than the allopathy medicines available in the market. As we can infer from our Comparative Docking Score tables that the potential affinities of ligand binding are more in case of Plant extracts of Triphala than that of affinities of medicines screened for the study. Hence, we can easily say that the extracts of medicinal plants are more effective for fighting against Alzheimer's than our easily available medicines prescribed by doctors. As we know the severity of this disease and many researches are going on daily basis on these medicinal plants which have remedy for AD and oxidative stress. We can conclude that we should prefer these herbal medications against AD than allopathy ones due to such research gap.

LIST OF FIGURES:

- **Fig 1-2** : W. Grodzicki and K. Dziendzikowska The Role of Selected Bioactive Compounds in the Prevention of Alzheimer's Disease 2020,9,229 3,
- **Fig3:** http://tinyurl.com/bdhwsryz
- **Fig 4:** http://tinyurl.com/tvshdzuk
- **Fig 5**: http://tinyurl.com/ydt6yyd2
- **Fig 6**: http://tinyurl.com/37t4bztu

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