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Network Pharmacology-guided Identification and Molecular Validation of Multi-Target Phytoconstituents from *Gmelina arborea* against Alzheimer's Disease

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Abstract

Alzheimer's disease (AD), one of the most prevalent neurodegenerative disorders, remains without a definitive cure due to its complex multifactorial pathogenesis. Conventional drug development strategies, targeting single pathway, have demonstrated limited success, necessitating a paradigm shift towards multi-target therapeutics. In this study, we systematically investigated the therapeutic potential of *Gmelina arborea* (GA), a medicinal plant used in traditional Indian medicine against dementia, using an integrated network pharmacology and molecular modeling approach. Phytoconstituents of GA (GAPC) were selected and screened for drug-likeness (DL), blood-brain barrier (BBB) permeability, and absorption, distribution, metabolism, excretion, and toxicology (ADMET) properties. Potential therapeutic targets of GAPC were predicted and cross-referenced with known AD-associated targets to construct a protein-protein interaction (PPI) network of common targets. Functional enrichment analysis revealed key aspects of gene ontology (GO) and pathways, including PI3K-Akt, MAPK, FoxO, Rap1, and Ras signaling pathways. Top ten core target genes (using topological analysis) were identified as AKT1, EGFR, ESR1, SRC, PTGS2, GSK3 β , MMP9, PARP1, KDR, and ABCB1. Molecular docking, molecular dynamics (MD) simulations, molecular mechanics, the Generalized Born Surface Area (MM-GBSA), principal component analysis (PCA), and free energy landscape (FEL) analysis confirmed strong, stable binding interactions, especially for verbascoside and martynoside. This study provides compelling evidence that GA can be targeted for AD treatment following experimental validation and lays the foundation for further wet lab investigations. It also presents the first integrated in silico

and network pharmacology analysis of GAPC's multi-target interactions in AD, offering mechanistic insights to guide future research.

Key Words: Acetylcholinesterase, Alzheimer's Disease, BACE1, Free Energy Landscape, *Gmelina arborea*, Martynoside, MM-GBSA, Molecular Docking, Molecular Dynamics Simulation, Multi-Target Therapy, Network Pharmacology, Neuroprotection, Principal Component Analysis, Phytoconstituents, Tau protein, Verbascoside

1. Introduction

Dementia affects over 55 million individuals worldwide, with more than 60% residing in low- and middle-income countries. Annually, approximately 10 million new cases are reported. This condition arises due to various diseases and brain injuries, with AD being the most prevalent form, accounting for 60–70% of cases. Globally, dementia ranks as the seventh leading cause of mortality and significantly contributes to disability and dependency among the elderly population [1]. Individuals diagnosed with AD experience short-term memory impairment, which hampers their ability to perform daily activities efficiently [2]. The exact mechanisms underlying AD-related pathological changes remain unclear. However, two key neuropathological hallmarks characterize AD: extracellular deposits of amyloid beta ($A\beta$) protein [3] and neurofibrillary tangles (NFT), which consist of hyperphosphorylated tau protein [4]. The accumulation of $A\beta$ is believed to interfere with cholinergic neurotransmission, reducing acetylcholine (ACh) levels [5]. Additionally, several hypotheses suggest that inflammation plays a crucial role in the pathogenesis of AD [6]. Furthermore, excessive production of reactive oxygen species (ROS) has also been linked to AD pathophysiology [7].

Current treatment options for AD focus on blocking acetylcholinesterase and $A\beta$ cleaving enzymes, as well as taking antioxidants to combat oxidative stress, which is a major factor to disease progression [8]. Current medicines, such as cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and N-methyl-D-aspartate (NMDA) receptor antagonists (memantine), generally provide symptomatic relief without affecting disease progression [9]. Additionally, newer drugs such as aducanumab (discontinued in November 2024), lecanemab, and donanemab (approved in July 2024) target amyloid plaques in the brain. However, these agents have demonstrated only modest clinical efficacy and are associated with significant side effects such as brain swelling and microhemorrhages, collectively referred to as Amyloid-Related Imaging Abnormalities (ARIA) [10]. Moreover, the genetic and environmental factors contributing to AD are not fully understood, further limiting development of novel treatments [11].

AD is a complex disorder involving multiple pathological mechanisms such as amyloid aggregation, tau pathology, and neuroinflammation; still the current drug discovery approaches involve developing a highly selective ligand that either activates or inhibits a particular target that has been shown to have an important role in the pathophysiology of a disease. This method relies on the notion that a ligand with a significant level of specificity for a single target is safe and effective, with few side effects [12]. The single-molecule, single-target approach has struggled to provide effective treatment, as focusing on just one pathway has shown limited success in AD.

The traditional ‘one gene, one drug, one disease’ model of drug discovery is being reconsidered in light of emerging insights from systems biology and network pharmacology. Polypharmacology (the study of drugs that target multiple biological pathways or receptors) provides a broader perspective by addressing multiple interconnected targets, which can enhance therapeutic efficacy and slow disease progression. Network pharmacology, a computational approach, investigates drug actions and their interactions with multiple therapeutic targets. This methodology is instrumental in identifying drug candidates and elucidating their mechanisms of action by analyzing their effects on multiple targets simultaneously [13]. Network pharmacology plays a crucial role in the development of herbal medicines, as natural phytoconstituents are known to influence multiple signaling pathways [14,15]. By systematically analyzing biological networks, this approach helps uncover drug mechanisms, predict potential targets, assess possible adverse effects, and facilitate drug discovery. Thus, unlike conventional methods that focus on single targets, network pharmacology emphasizes disease-associated networks and seeks ligands capable of modulating multiple therapeutic targets [16]. Phytoconstituents that act on multiple receptors hold great promise for drug discovery. Experimental research on AD has shown that many phytoconstituents (for instance, curcumin, resveratrol, berberine, huperzine A, ginsenosides, galantamine, bacopaside A and B, etc.) exert beneficial effects through various mechanisms, such as reducing oxidative stress-induced neuronal damage, lowering neuronal inflammation, and preventing mitochondrial dysfunction [17]. Network pharmacology helps predict potential phytoconstituent-target interactions, which are then validated by molecular docking through binding affinity analysis. MD simulations further assess the stability and behavior of these interactions over time, collectively enhancing the reliability of network pharmacology in drug discovery [18].

GA, a fast-growing deciduous tree belonging to family lamiaceae, is an important medicinal plant in the Indian systems of medicine and is reported to possess cardiogenic, anti-

inflammatory, anti-diabetic, cytotoxic, antimicrobial, antioxidant, wound healing, and gastro-protective properties. It is also used in Ayurvedic medicine to treat fever, emaciation, heart and neurological diseases, enhance memory, and alleviate giddiness [19], [20]. Moreover, GA is an essential ingredient in traditional Ayurvedic polyherbal preparations mainly *Dashmularishta*, and *Chyawanprash* [21]. *Dashmularishta* is an Ayurvedic polyherbal formulation prepared in the form of fermented decoction. This is much useful in the treatment of long period AD, hemiplegia, and other diseases[22]. Similarly, in Ayurveda, *Chyawanprash* is classified under the category of *Rasayana*, which aims at maintaining physique, vigor and vitality, while delaying the ageing process [23]. It is also recommended as a useful remedy for the management of AD owing to its antioxidant effect, pro-cholinergic action and/or anti-amnesic potential[24].

Various secondary metabolites have been attributed to different pharmacological activities of GA [19,25–31]. Moreover, preliminary research shown positive effects of GA against AD [32–34]; but the precise mechanism of action of various GAPC against AD is yet unknown. The complex molecular interactions of these GAPC in AD treatment require further investigation. This study aims to identify the major GAPC that can modulate multiple therapeutic targets involved in AD pathogenesis using network pharmacology and subsequent validation via molecular docking, MD simulations, and MM-GBSA, PCA and FEL analysis. The study protocol is outlined in Figure. 1. This study provides the first comprehensive computational investigation into the anti-Alzheimer's potential of GA, integrating network pharmacology, molecular docking, MD simulations, MM-GBSA, PCA, and FEL analysis. By exploring the multitarget interactions of lesser-known phytoconstituents with key Alzheimer's-related proteins, the research offers novel mechanistic insights and a strong in silico foundation for future experimental validation of GA as a potential therapeutic candidate.

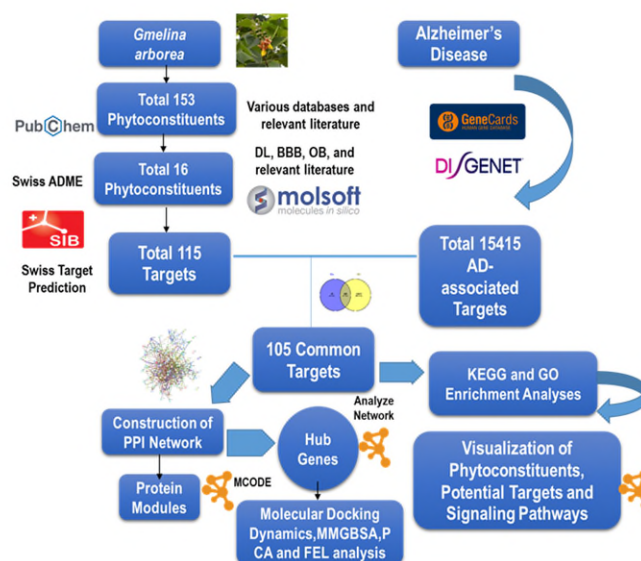


Figure 1: The study protocol from network pharmacology to free energy landscape

Figure 1: The study protocol from network pharmacology to free energy landscape (The logos/symbols shown in the diagrams are used solely for illustrative purposes and are the property of respective organizations)

2. Materials and Methods

We utilized multiple online databases and conducted a deep literature review to compile a list of GAPC. Subsequently, we identified the potential targets of GAPC. In parallel, targets associated with AD were gathered from various databases. The intersection of these datasets yielded common targets, which were selected for further analysis. Protein–protein interaction (PPI) networks, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and GO analysis were then performed to explore their potential involvement in AD pathogenesis. Hub genes (identified using Cytoscape v3.10.3) and promising phytoconstituents were subjected to molecular docking. Further evaluation was carried out like MD simulations to assess the stability of the complexes, MM-GBSA to estimate binding free energies, PCA to examine major conformational changes, and FEL analysis to visualize stable states and transition pathways.

2.1. Identification and Screening of Targets of GAPC

GAPC were collected from IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics), a curated database of Indian medicinal plants and their phytochemicals [35], KNApSAcK Family Database [36,37], and Chemical Entities of Only Biological Interest [38]

all accessed on 05th February, 2025. For this, GA was used as the keyword in all four databases. Only unique GAPC were kept for further study while discarding duplicate ones. These GAPC were then screened for ADMET, DL, BBB penetration and oral bioavailability (OB) by accessing the Swiss ADME database [39], and Molsoft LLC [40] (accessed on 18th February 2025). GAPC with DL values ≥ 0.18 , OB $\geq 30\%$, and BBB ≥ -0.3 were identified as potential GAPC and kept for further study. In addition, the analysis included many active compounds that did not match the screening criterion but had been described in the literature. Then, all the targets of these screened GAPC with a probability ≥ 0.4 were predicted using the Swiss Target Prediction database [41] (accessed on 20th February 2025).

2.2. Identification of AD-associated Targets

AD-associated targets were mined from two resources: the GeneCards database [42], and the DisGeNET database [43] (accessed on 24th February 2025). Finally, after merging and removing duplicate entries, a final list of AD-associated targets was prepared.

2.3. Identification of Targets at the Intersection of AD and GAPC

The Venny 2.1 web tool [44] (accessed on 27th February 2025) was used to map the targets of AD and GAPC to find common targets [38]. The common targets were indicated by the overlapped area (Figure 2).

2.4. Construction and Analysis of the PPI Network

The STRING database [45] (accessed on 27th February 2025) is a comprehensive resource that compiles both known and predicted protein associations, encompassing physical interactions as well as functional links. We leveraged STRING to analyze the relationship between targets at the intersection of AD and GAPC. A PPI network was constructed under the following parameters: the species was set to Homo sapiens, and the confidence threshold was defined as "medium" (≥ 0.4). This confidence score of 0.4 was chosen because it effectively balances sensitivity and specificity, making it a reliable criterion for identifying meaningful protein interactions. The PPI network was exported to tsv. format file and then this file was imported into Cytoscape [46]. The topological analysis was then performed using network analyzer tool in Cytoscape. The core features of the network's nodes were estimated using three topological parameters: degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC). The degree refers to the total number of direct links a node has within a network and is often used to determine its significance. Likewise, BC, CC assess a node's role based on its ability to connect with other nodes or facilitate information transfer between them. Core targets were identified on the basis of these topological parameters. The targets above the median of the degree value, BC, and CC were selected as core targets. The MCODE plugin in Cytoscape

was used for clustering analysis to identify key functional modules in PPI. The analysis was performed with a degree cut-off of 2 and a node score cut-off of 0.2. The Haircut option was enabled, while Fluff was disabled, ensuring refined clusters. A K-core of 2 was set, and the maximum depth from the seed node was set to 100, optimizing the identification of highly interconnected protein clusters relevant to AD pathogenesis.

2.5. Functional Enrichment and Pathway Analysis

All common identified therapeutic targets were analyzed for GO and KEGG pathway enrichment using the STRING database (accessed on 3rd March, 2025) to determine relevant pathways and GO terms categorized under biological process (BP), molecular function (MF), and cellular component (CC). Only pathways and GO terms meeting the significance threshold of FDR value less or equal to 0.05 were considered for further analysis. To illustrate the results, STRING database online tool was employed to generate bubble charts for signaling pathways and GO categories. Finally, the interaction network linking GAPC, target proteins, and signaling pathways was constructed using Cytoscape[46,47].

2.6. Virtual Screening of Compounds using Molecular Docking

From total 16 GAPC (Table 2), extensively reported ones were excluded from further studies that is molecular modelling, as their anti-AD potential has been extensively reported in previous studies. Instead, docking was performed on lesser-explored GAPC to identify novel therapeutic candidates. In this context, the binding affinity of martynoside, verbascoside, quercetagenin, and tyrosol to AD targets was evaluated using AutoDock v1.5.7 [48]. While the top ten identified common targets (Table 3 and Supporting File Sheet 4) exhibit therapeutic potential, it is crucial to recognize that AD is a multifactorial disorder requiring a comprehensive approach that addresses multiple pathological pathways. Consequently, for molecular modelling studies, four receptors (GSK3B, BACE1, MAPT, and AChE) were selected (Table 1). While only GSK3B from the top 10 core targets was taken forward for molecular modeling, our selection of AChE, BACE1, and MAPT was driven by their direct roles in key pathological processes of AD, cholinergic dysfunction, amyloidogenesis, and tau aggregation, respectively. Notably, GSK3B was identified in MCODE cluster 1, and AChE, BACE1, and MAPT were clustered together in MCODE cluster 2, further supporting their critical functional relevance. Prioritizing these targets provides a mechanistically diverse and clinically meaningful strategy for identifying multi-target therapeutics in AD.

The PubChem database was used to get the 3D structures of ligands and Open Babel v2.4.1 converted them from sdf to.pdb [49,50]. Avogadro was used to optimize the structures by reducing the energy using the MMFF94 force field and Steepest Descent algorithm for 5000

steps. The optimization stopped when the energy difference was less than 0.1, and the structures were stored as.pdb files. The 3D crystal structures of the receptors were obtained from RCSB-PDB database (Table 1) [51]. The receptors had polar hydrogen and Kollman charge added after removing water and het atoms. The protein structures were saved as. pdbqt format. Biovia Discovery Studio and Schrodinger Maestro tool was used to visualize the protein–ligand complexes after docking by AutoDock Vina [48,52].

Table 1: Information about the target protein.

Sr. No.	Target	PDB_id	Resolution (Å)	UniProt entry
1	GSK3B	4ACC	2.21	P49841
2	BACE1	4IVT	1.60	Q8IXC6
3	AChE	4EY7	2.35	P22303
4	MAPT	5ML5	1.90	P10636

2.7. Molecular Dynamics Simulation

The best docking score's hit phytoconstituent out of four on each receptor (4ACC,4IVT,4EY7 and 5ML5) with the reference ligand structure complex were selected for MD simulation using the Desmond module 2020.1. A 100 ns MD simulation was conducted on each ligand-protein complex within the Desmond module, employing periodic boundary conditions. To immerse the protein-ligand complex in a SPC (simple point charge) explicit water model, an orthorhombic box was generated using the system builder tool. The distance between the complex and the box wall was maintained at a minimum of 10 Å for every atom in the ligand-protein complex. In order to neutralize the system, a suitable number of counter ions (sodium and chloride) were introduced, while the addition of 150 mM NaCl to the simulation box emulated physiological or isosmotic conditions. The energy of the system was minimized to ensure the proper alignment of the protein structure within the simulation boundaries. This minimization process involved 000 iterations and utilized an OPLS3e force field, with a convergence criterion of 1 kcal/mol/Å. Before the actual production run of the simulation, a predefined equilibration protocol was executed. The MD simulation took place under NPT conditions, maintaining a temperature of 300° K and a pressure of 1.013 bar. To regulate temperature and pressure, a Nose-Hoover-Chain thermostat and Martyna-TobiasKlein barostat were employed. The energy and structure of the system were saved in the trajectory at intervals of 10 ps, resulting in a total of 1000 frames in the trajectory.[53]

2.8. MM-GBSA Analysis

MM-GBSA analysis was performed to determine and calculate the free energies of the ligands and complexes by the Schrodinger Maestro suite. An OPLS-AA forcefield-based molecular mechanics energies were used for the polar solvation energies along with the variable dielectric generalized born 2.0 (VSGB) solvation model. Incorporation of Solvent-accessible surface area and Van der waals interactions was performed by integration of non-polar interactions with the use of Prime-MM-GBSA analysis. In the present study, 100 from 1000 snapshots derived in the 100 ns run of the compounds were taken for MM-GBSA calculation of protein-ligand complexes[54].

2.9. Principal Component Analysis

PCA was performed to recognize how ligand binding interferes the dynamics of Ca atoms by decomposing the Ca RMSD. With PCA, it is possible to interpret the connections between the various Ca conformations of Alzheimer in the complex simulated systems that were achieved during trajectory and used in the Bio3D-R statistics tool for protein structure comparison. In short, VMD was used to import the Desmond trajectory (.dtr) files and rewrite them as trajectory dcd files. The PCA analysis required a list of numerical coordinates in x, y, and z coordinates, it was created by combining the dcd files that were loaded into R Studio and the PDB file of the reduced structure with the Ca atoms for trajectory frames. [55–57]

2.10. Free Energy Landscape

The FEL was used to explore three-dimensional conformational sampling. It was generated using Python 3 with the NumPy library. RMSD, and Rg were used to create FEL energy and calculate the joint probability distribution in three dimensions.[58]

3. Results

3.1 GAPC and AD-associated Targets

Various databases were used to download different GAPC, supplemented by additional compounds obtained through a literature search. After eliminating duplicates (as well as commonly found fatty acids, sugars, and amino acids), a total of 153 compounds (Supporting File-Sheet 1) were retained for further screening.

3.2 Prediction of ADMET Properties

The ADMET properties including BBB were predicted using Molsoft, and SwissADME tool. Umbelliferone, tyrosol, martynoside, and verbascoside were also included for further studies based on the literature survey. Thus, we shortlisted total 16 GAPC (Table 2 and Supporting File-Sheet 2).

Table 2: DL, BBB Permeability, and Bioavailability Score of GAPC

Sr. No.	Chemical Class	GAPC	DL	BBB	OB Score
1	Phenylpropanoid glycoside	Martynoside	0.44	1	0.17
2	Phenylethanoid glycoside	Verbascoside	0.51	1.18	0.17
3	Coumarin	Umbelliferone	-0.65	3.83	0.55
4	Coumarin	7-Geranyloxy-5-methoxycoumarin	0.29	4.25	0.55
5	Flavonoid glycoside	Luteolin-7-glucuronide	0.71	1.61	0.11
6	Flavonoid glycoside	Apigenin 7-glucuronide	0.67	1.66	0.11
7	Flavonol	Kaempferol	0.50	2.78	0.55
8	Flavonol	Quercetin	0.52	2.55	0.55
9	Flavonol	Quercetagenin	0.39	2.35	0.55
10	Flavone	Apigenin	0.39	2.97	0.55
11	Flavone	Luteolin	0.38	2.75	0.55
12	Phytosterol	Campesterol	0.59	3.99	0.55
13	Phytosterol	Stigmasterol	0.22	3.93	0.55
14	Phytosterol	beta-Sitosterol	0.29	4.25	0.55
15	Phytosterol	Stigmasterol	0.29	4.25	0.55
16	Phenylethanoid	Tyrosol	-1.16	4.04	0.55

3.3. Target Identification of GAPC and AD

All the targets of above 16 GAPC were predicted by inputting individual phytoconstituent in the Swiss Target Prediction database. Targets with a probability ≥ 0.4 were saved, rest were discarded. Using this filter total 115 targets were saved (Supporting File- Sheet 3). Now, using GeneCards and DisGeNET databases, total 15519 targets for AD were saved for subsequent analysis (Supporting File-Sheet 3).

3.4. Targets Overlapping between GAPC and AD

115 targets of GAPC and 15519 targets involved in AD were submitted to Venny 2.1 software to get common targets (Figure 2). Total 105 common targets were found (Supporting File-Sheet 3), which were analysed using STRING database. A network of common targets and GAPC is shown in Figure 5.

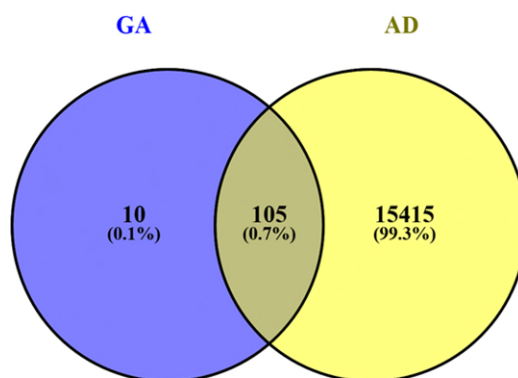


Figure 2: Venn Diagram showing the GAPC targets, targets-associated with AD, and common targets

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3.5: PPI Network Visualization and Analysis

The STRING database was utilized to examine interactions among 105 potential therapeutic targets. To visualize the PPI network, Cytoscape was employed. The constructed PPI network consisted of 105 nodes and 623 edges, with an average node degree of 11.9 (Figure 3). In this network, nodes represent individual proteins, while edges signify interactions between them. A higher degree value indicates a more crucial role of a protein within the network. Based on the applied selection criteria, 10 hub genes (core targets) were identified: AKT1, EGFR, ESR1, SRC, PTGS2, GSK3B, MMP9, PARP1, KDR, and ABCB1 (Table 3). In the PPI network, these genes are regarded as key targets because of their superior network topological metrics, including degree, BC, and CC.

The MCODE plugin generated eight clusters (Figure. 4a-4h). Nine out of ten core targets were clustered within the top two functional modules (PTGS2, ABCB1, KDR, PARP1, GSK3B, MMP9, SRC, ESR1, and AKT1), further indicating their pivotal role in mediating the effects of GA against AD (Supporting File- Sheet 9). This demonstrates that these clusters may be the most relevant to AD treatment. Cluster 1 contains 14 nodes and 69 edges with a score of 10.61. The seed node of this cluster is CCNB1 (Cyclin B1), which regulates cell cycle progression; its dysregulation may contribute to neuronal cell death and neurodegeneration in AD[59]. Cluster 2 contains 17 nodes and 60 edges with a score of 7.5. The seed node of this cluster is PIK3R1 (Phosphoinositide-3-Kinase Regulatory Subunit 1), a key player in

PI3K/AKT signaling; impaired function leads to neuroinflammation, oxidative stress, and tau hyperphosphorylation in AD[60]. Cluster 3 contains 4 nodes and 6 edges with a score of 4. The seed node of this cluster is ALOX5 (Arachidonate 5-Lipoxygenase) which is involved in neuroinflammation and increased activity is linked to A β aggregation and cognitive decline [61]. Cluster 4 contains 4 nodes and 6 edges with a score of 4. The seed node of this cluster is CA7 (Carbonic Anhydrase 7). CA7 regulates pH and ion homeostasis. Its dysregulation may contribute to oxidative stress and neuroinflammation in AD [62]. Cluster 5 contains 4 nodes and 5 edges with a score of 3.33. The seed node of this cluster is GLO1 (Glyoxalase 1). It detoxifies reactive aldehydes, and its reduced activity leads to accumulation of advanced glycation end-products (AGEs), contributing to neurodegeneration[63]. Cluster 6 contains 3 nodes and 3 edges with a score of 3. The seed node NR1H3 (Liver X Receptor Alpha, LXR α) modulates lipid metabolism and amyloid clearance; dysfunction is associated with increased A β deposition[64]. Cluster 7 contains 3 nodes and 3 edges with a score of 3. The seed node AHR (Aryl Hydrocarbon Receptor) regulates neuroinflammation and oxidative stress; aberrant signaling may exacerbate AD pathology [65]. Cluster 8 contains 6 nodes and 7 edges with a score of 2.8. The seed node AURKB (Aurora Kinase B) controls mitotic spindle formation. Its dysregulation is implicated in neuronal apoptosis and tau pathology in AD [66].

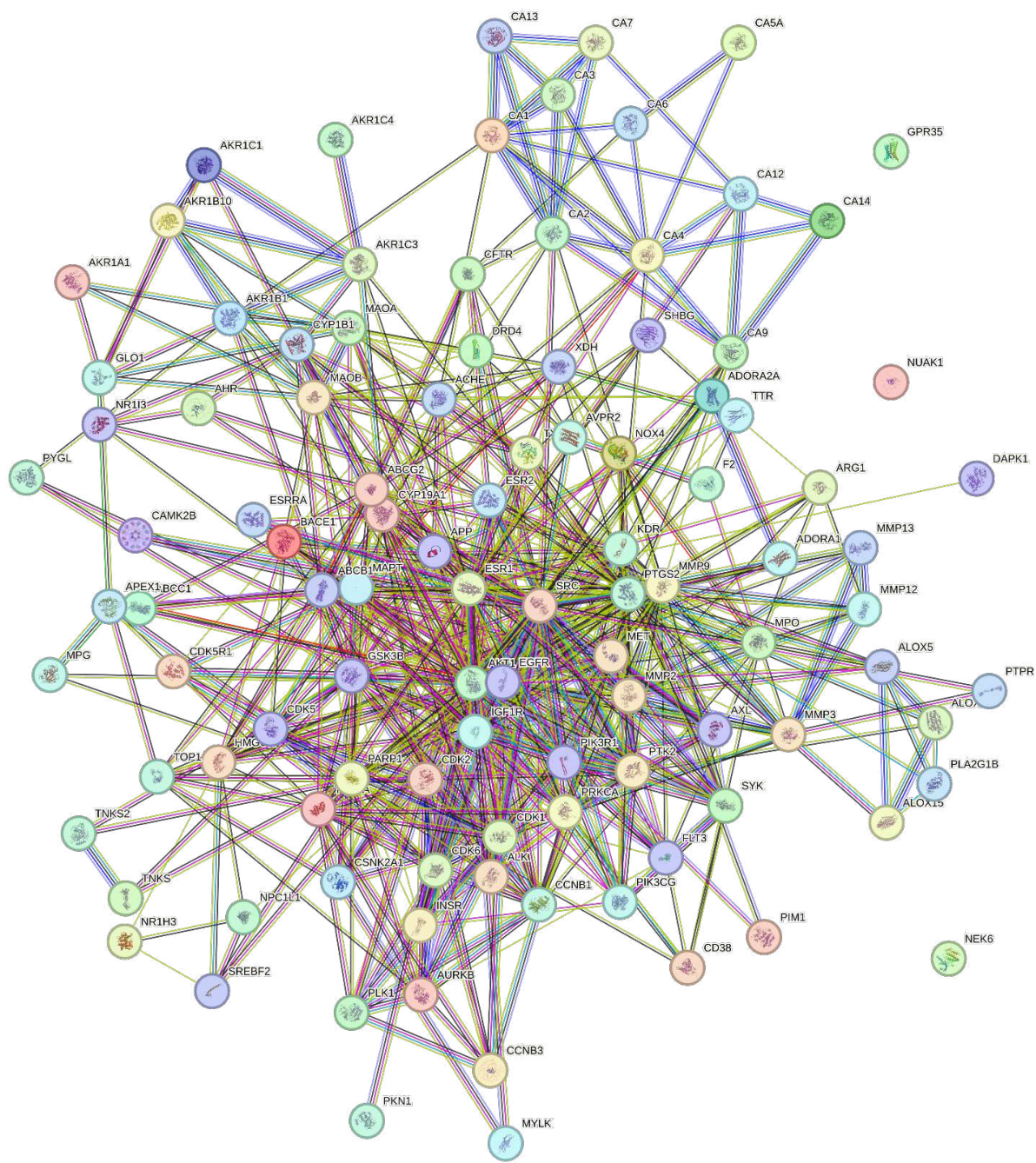


Figure 3: The PPI network represents common target interactions, with proteins as circular nodes and edges denoting connections. Purple edges indicate curated database interactions, pink represent experimental data, while green, red, and blue signify predictions from gene neighborhood, fusions, and co-occurrence. Yellow and black edges denote text mining and co-expression interactions, respectively.

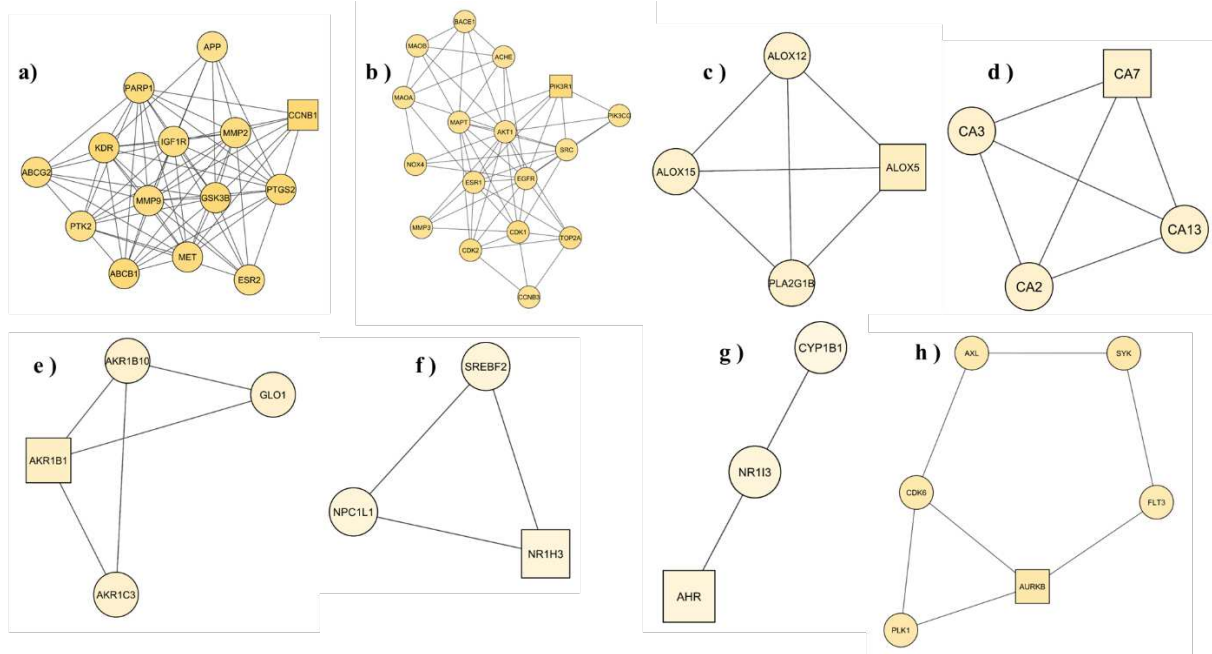


Figure 4a-4h: MCODE Cluster Analysis

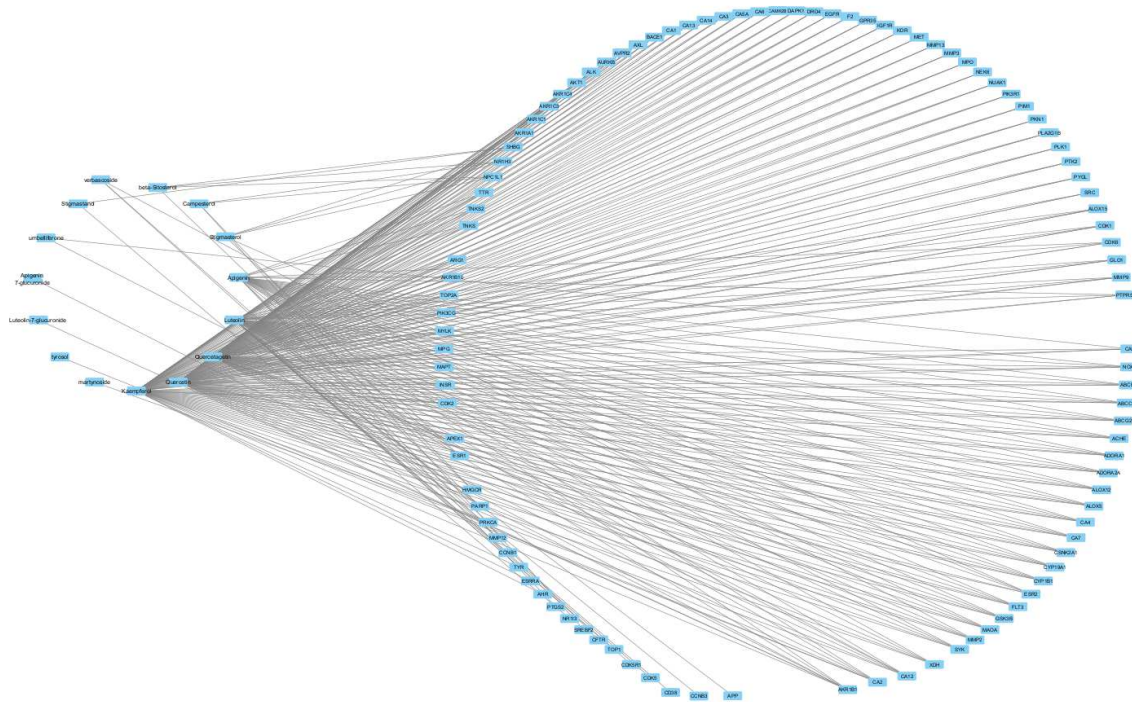


Figure 5: Interaction network of 15 GAPC (left small circle) and 104 common target genes (right bigger circle). Lines represent the interaction between left circle nodes and right circle nodes.

nodes. 7-Geranyloxy-5-methoxycoumarin was deleted from the network as it was associated with single target which was also not involved with other GAPC.

Table 3: The top 10 intersection targets with values of Degree, Betweenness Centrality, and Closeness Centrality above the median

Uniprot ID	Protein Name	Gene Name	Degree	Betweenness Centrality	Closeness Centrality
P31749	RAC-alpha serine/threonine-protein kinase	AKT1	53	0.138458	0.647436
P00533	Epidermal growth factor receptor	EGFR	46	0.097849	0.612121
P03372	Estrogen receptor	ESR1	45	0.125712	0.627329
P12931	Proto-oncogene tyrosine-protein kinase Src	SRC	41	0.09298	0.597633
P35354	Prostaglandin G/H synthase 2	PTGS2	40	0.096397	0.60119
P49841	Glycogen synthase kinase-3 beta	GSK3B	38	0.060058	0.587209
P14780	Matrix metalloproteinase-9	MMP9	36	0.039422	0.583815
P09874	Poly [ADP-ribose] polymerase 1	PARP1	32	0.044909	0.558011
P17948	Vascular endothelial growth factor receptor 1	KDR	26	0.023197	0.548913
P08183	ATP-dependent translocase ABCB1	ABCB1	23	0.021748	0.534392

3.6: GO Enrichment Analysis

To explore the biological mechanisms of GA in AD, GO enrichment analysis was conducted on 105 potential therapeutic targets associated with AD using STRING database online tool. The analysis was categorized into three distinct areas: BP, MF, and CC. We obtained total 844 BP, 119 MF, and 77 CC terms (Supporting File-Sheet 5-7). Out of these, top ten terms based on number of genes and FDR value, were visualized in the bubble plot (Figure. 6a-6c). The bubble size and color indicate the enrichment gene and FDR value, respectively. Our analysis revealed that the main enriched BP categories were the cellular process, metabolic process, biological regulation, response to stimulus, cellular metabolic process, regulation of biological process, regulation of cellular process, organic substance metabolic process, cellular response to stimulus, and primary metabolic process. The main enriched MFs were binding, ion binding, catalytic activity, protein binding, organic cyclic compound binding, heterocyclic compound binding, cation binding, metal ion binding, anion binding, and small molecule binding. CC analysis revealed that the cellular anatomical entity, organelle, intracellular anatomical structure, membrane-bounded organelle, cytoplasm, intracellular organelle, intracellular membrane-bounded organelle, membrane, nucleus, and cell periphery were the most enriched CC terms.

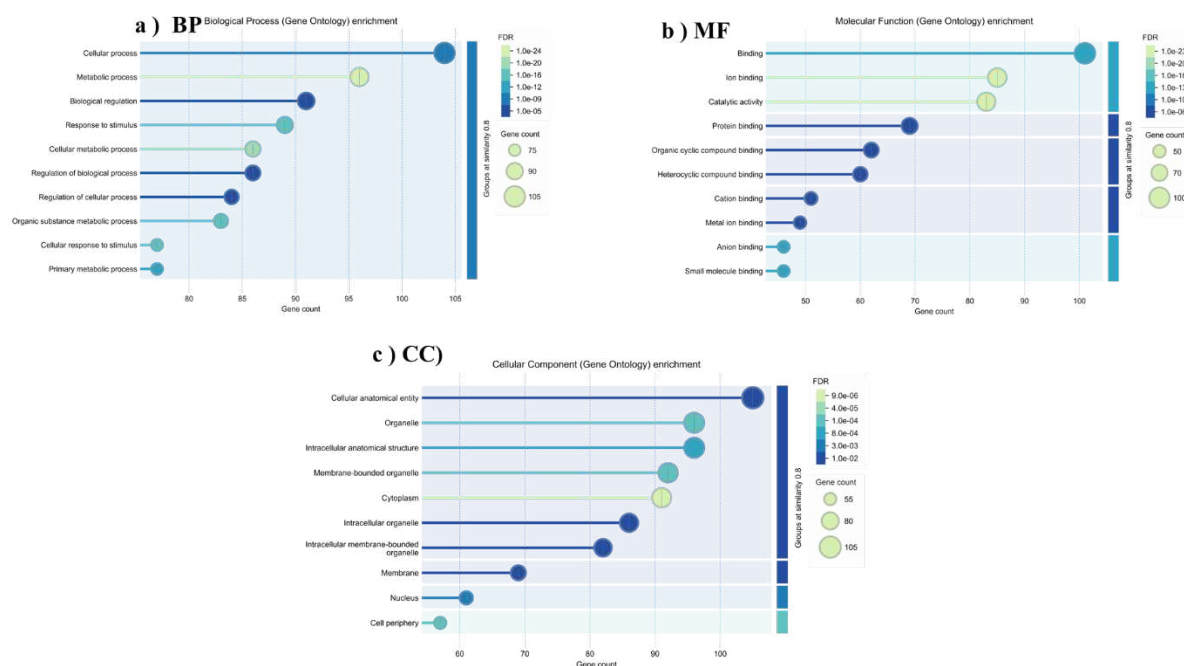


Figure 6: Enrichment analysis of GO function (a: BP, b: MF, and c: CC)

3.7. KEGG Pathway Enrichment Analysis

Pathways of potential therapeutic targets for the treatment of AD were identified through KEGG pathway enrichment analysis. Using the STRING database, we obtained 140 signaling pathways (Supporting File-Sheet 8). We plotted the top 20 signaling pathways in a bubble graph

(Figure 7) sorted by their FDR values and then applying high to low filter for the number of genes. The results showed that the key targets were enriched in the serotonergic synapse, human cytomegalovirus infection, Alzheimer disease, microRNAs in cancer, and pathways in cancer. To further analyze the relationship between 16 GAPC, 105 common therapeutic targets, and top 20 signaling pathways, we constructed a visual network using Cytoscape (Figure 8). The visual network comprised 144 nodes and 583 edges.

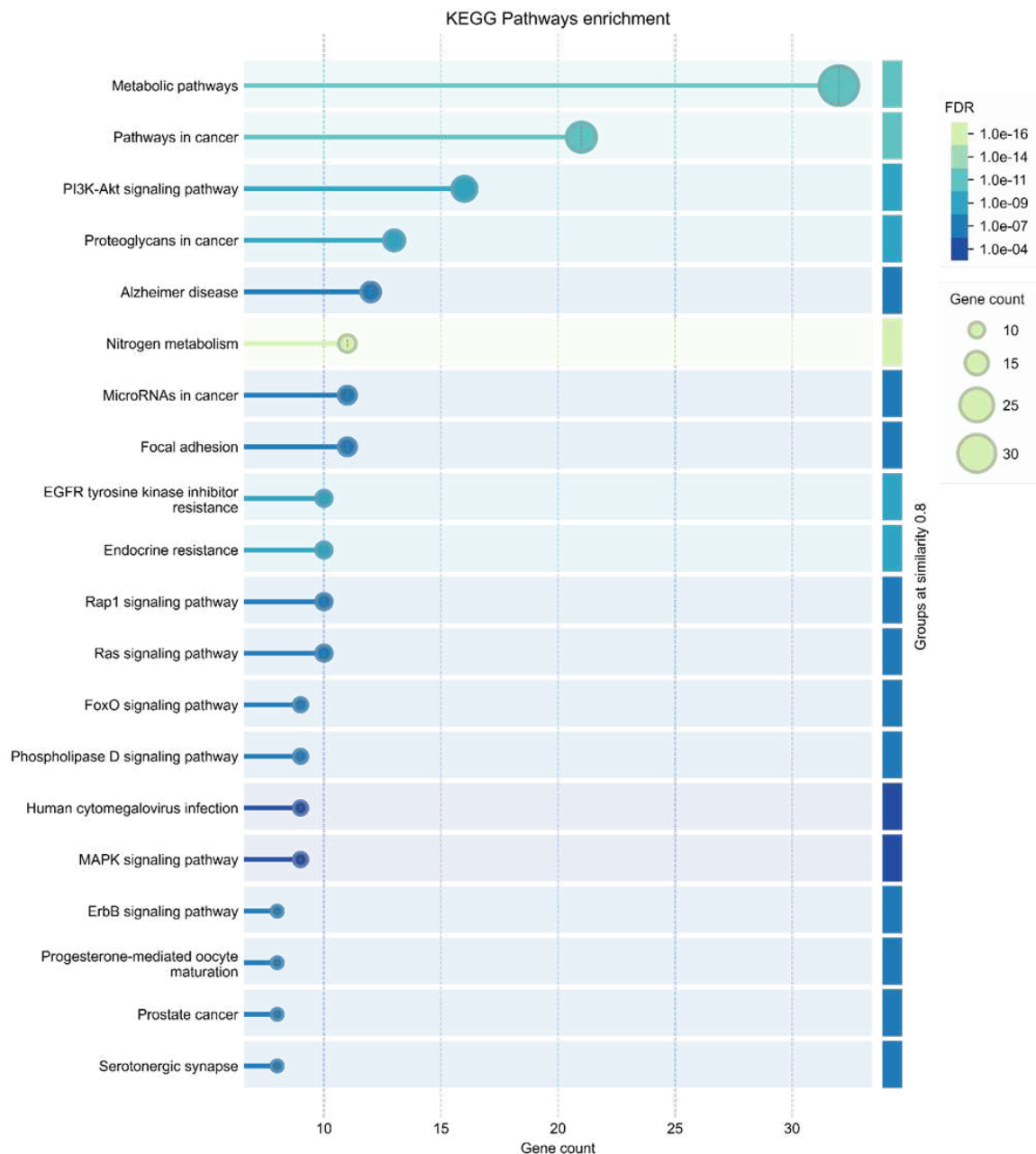


Figure 7: Enrichment analysis of KEGG pathway

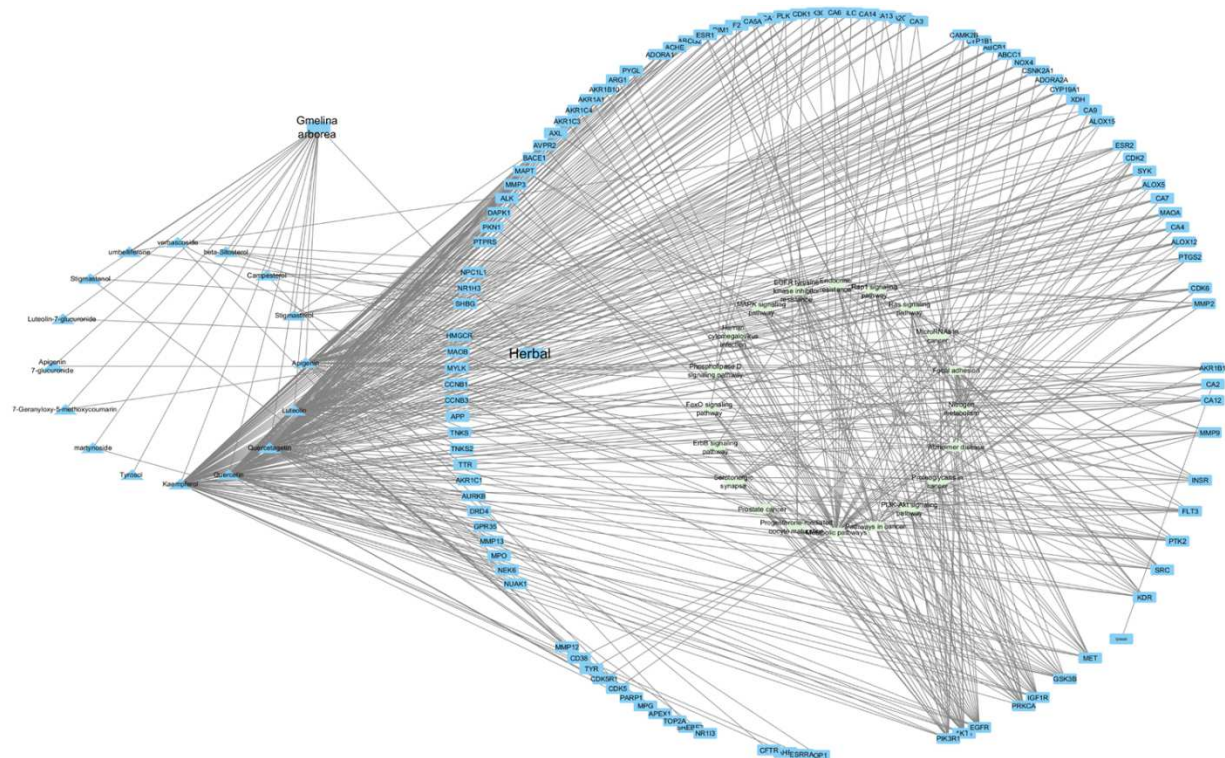


Figure 8: A network of GAPC, common targets, and signaling pathways. The left outer circle shows GAPC; inner circle shoes signaling pathways, and outer biggest circle indicates common targets.

3.8. Molecular Docking

Molecular docking was conducted to assess the binding affinities of four phytoconstituents, verbascoside, martynoside, quercetagetin, and tyrosol, against four AD-associated targets: GSK3 β (4ACC), BACE1 (4IVT), AChE (4EY7), and MAPT (5ML5). Verbascoside showed the highest binding affinity toward AChE (-13.618 kcal/mol) and GSK3 β (-12.029 kcal/mol), outperforming their respective co-crystal ligands (-17.881 and -5.176 kcal/mol) respectively. Martynoside exhibited strong docking scores with MAPT (-10.689 kcal/mol) and AChE (-13.966 kcal/mol). Quercetagetin showed moderate affinity, especially toward AChE (-12.487 kcal/mol), while tyrosol displayed the weakest interactions across all targets as shown in Table 4. The interaction profiles revealed that all phytoconstituents engaged in hydrogen bonding, polar, and hydrophobic interactions with key active-site residues, such as ARG-144 and ASP-200 (4ACC), GLN-73 and PHE-108 (4IVT), TYR-124 and SER-293 (4EY7), and ALA-51 and GLY-170 (5ML5) as shown in figure 9-12. These interactions suggest varying degrees of binding strength and specificity, with verbascoside and martynoside emerging as the most promising ligands. Detailed results are presented in Table 4 and Figure 9.

Table 4: Binding energy of eight active components and positive control drugs.

Target (PDB ID)	Compound	Docking Score (kcal/mol)	Interaction Types	Key Residues Involved
GSK3β (4ACC)	Verbascoside	-12.029	H-bond, Charged, Hydrophobic	ILE-65, VAL-135, ARG-144, LYS-183, ASP-200
	Martynoside	-11.429	H-bond, Charged, Hydrophobic	ILE-62, VAL-135, ASP-200
	Quercetagenin	-6.796	H-bond, Charged, Hydrophobic	VAL-135, ASP-200
	Tyrosol	-6.068	H-bond, Charged, Hydrophobic	VAL-135, ASP-200
	Reference Ligand	-5.176	H-bond, Charged, Hydrophobic	LYS-85, TYR-134, THR-138
BACE1 (4IVT)	Verbascoside	-8.193	H-bond, Polar, Hydrophobic	GLY-11, GLN-73, PHE-109, TYR-198, THR-232
	Martynoside	-7.277	H-bond, Polar	GLN-73, GLY-230
	Quercetagenin	-4.263	H-bond, Polar, Hydrophobic	ASP-32, GLN-73, PHE-108
	Tyrosol	-4.196	H-bond, Polar, Hydrophobic	ASP-32, PHE-108
	Reference Ligand	-9.250	H-bond, Polar, Pi–Pi stacking	GLN-73, PHE-108, ASP-228
AChE (4EY7)	Verbascoside	-13.618	H-bond, Polar, Charged	ASP-74, ASN-283, SER-293
	Martynoside	-13.966	H-bond, Hydrophobic, Pi–Pi stacking	TRP-86, PHE-295
	Quercetagenin	-12.487	H-bond, Polar, Charged, Hydrophobic	ASP-74, TYR-124, SER-293, ARG-296, TYR-341
	Tyrosol	-5.653	H-bond, Polar, Pi–Pi stacking	TYR-124, ARG-296, TYR-341
	Reference Ligand	-17.881	H-bond, Hydrophobic, Pi–Pi stacking	TRP-86, TRP-286, PHE-295, TYR-337
MAPT (5ML5)	Verbascoside	-9.310	H-bond, Polar	VAL-30, ALA-51, ASP-112, GLY-170
	Martynoside	-10.689	H-bond, Polar, Charged	VAL-30, ASP-112, GLY-170
	Quercetagenin	-8.811	H-bond, Polar, Hydrophobic	ALA-51, LYS-53, GLY-170
	Tyrosol	-5.341	Charged	LYS-53
	Reference Ligand	-12.093	H-bond, Hydrophobic, Pi–Pi stacking	LYS-53

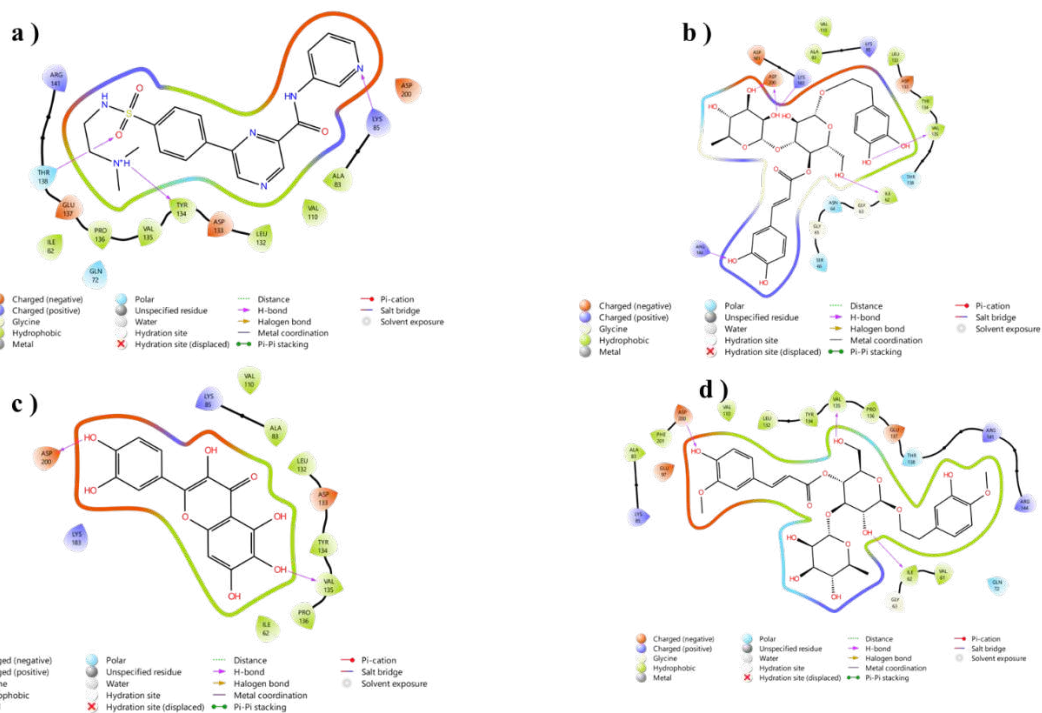


Figure 9: Ligand-Docking Interaction_4ACC a) Co_Crystal Ligand b) Verbacoside c) Quercetagetin d) Marytoniside

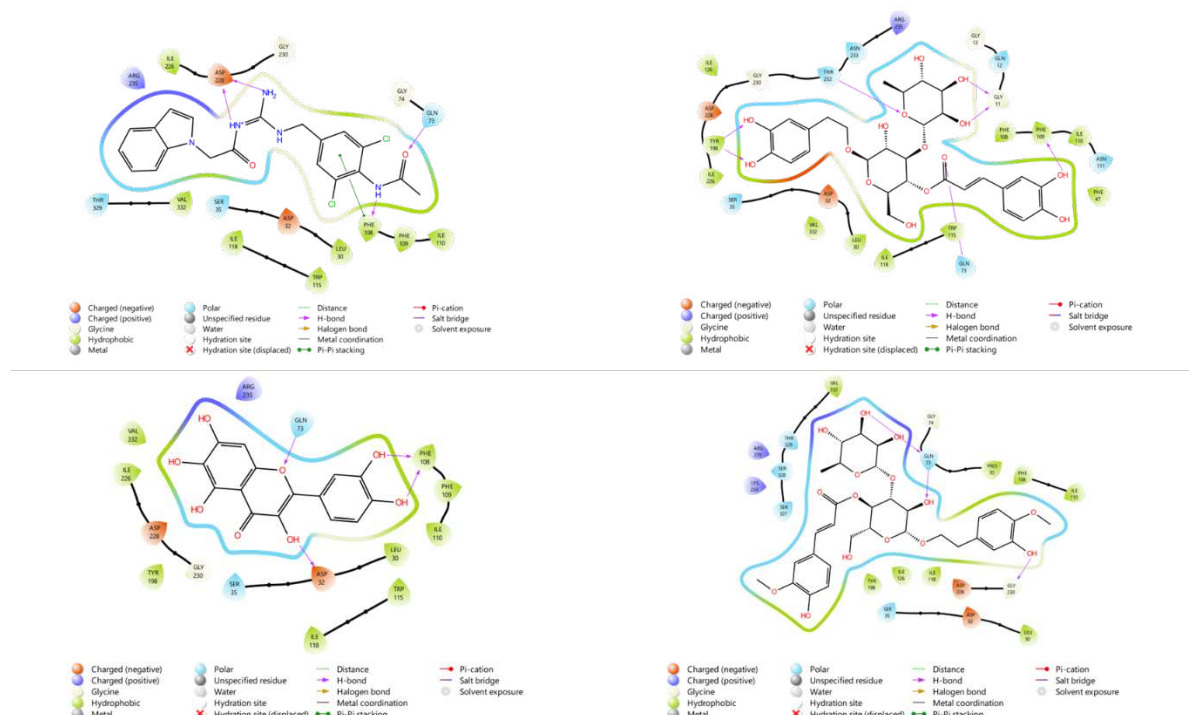


Figure 10: Ligand-Docking Interaction_4IVT a) Co_Crystal Ligand b) Verbacoside c) Quercetagetin d) Marytoniside

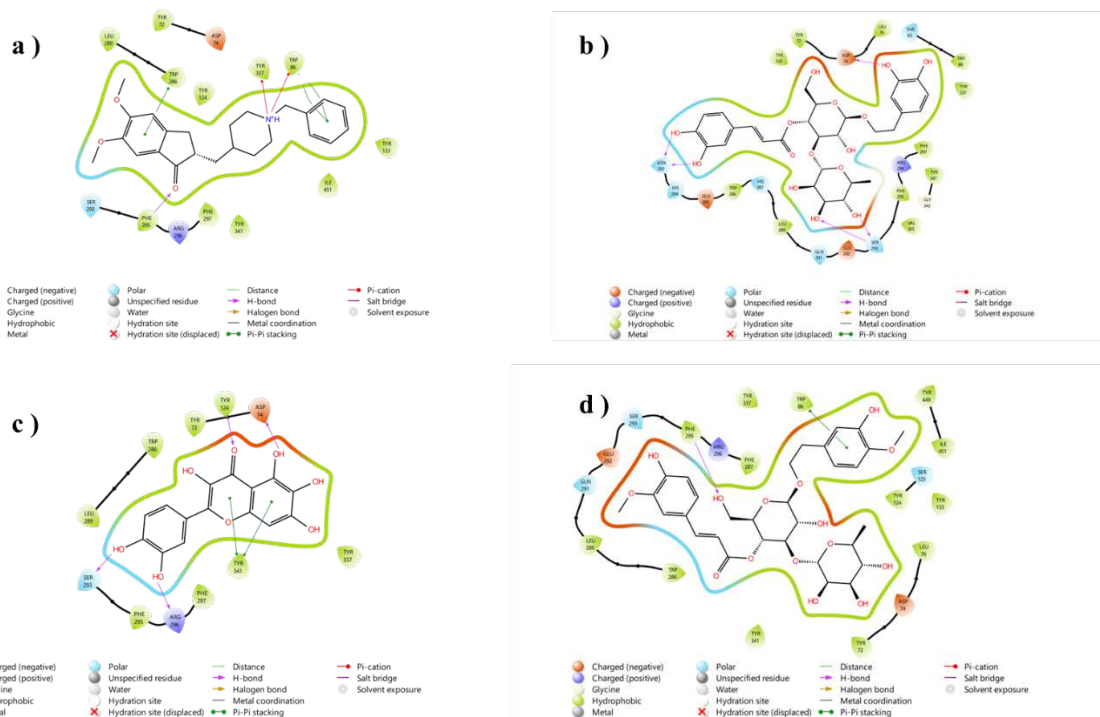


Figure 11: Ligand-Docking Interaction_4EY7 a) Co_Crystal Ligand b) Verbacoside c) Quercetagetin d) Marytoniside

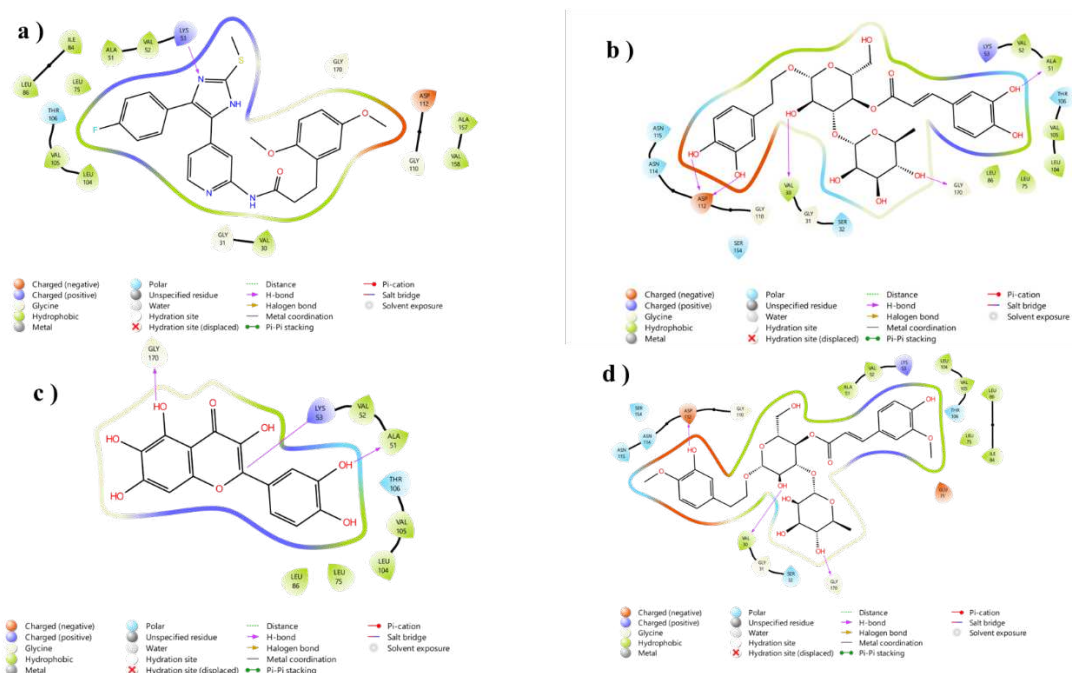


Figure 12: Ligand-Docking Interaction_5ML5 a) Co_Crystal Ligand b) Marytoniside c) Verbacoside d) Quercetagetin

3.9. Molecular Dynamics

MD simulations were carried out for 100 ns to assess the dynamic stability of the ligand–protein complexes. In the AChE (4ACC) complex shown in figure 13, the RMSD of the verbacoside–

protein complex remained stable around 2.0–2.5 Å for both C α atoms and the backbone, comparable to the co-crystal reference ligand, indicating a stable binding throughout the simulation. For the BACE1 (4IVT) system shown in figure 14, the verbascoside complex exhibited RMSD fluctuations between 1.8–2.3 Å, closely matching the reference ligand and suggesting a robust and consistent interaction. In the MAPT (4EY7) complex shown in figure 15, verbascoside maintained RMSD values around 2.1–2.6 Å, demonstrating minimal deviation and stable complex formation over time. Regarding the GSK3 β (5ML5) complex shown in figure 16, marytonoside maintained stable RMSD values between 2.0–2.7 Å for both C α atoms and the backbone, similar to the co-crystal ligand, reflecting a reliable binding stability. Collectively, the MD simulation results corroborate the docking findings, confirming that verbascoside and marytonoside form dynamically stable complexes with their respective AD-related targets.

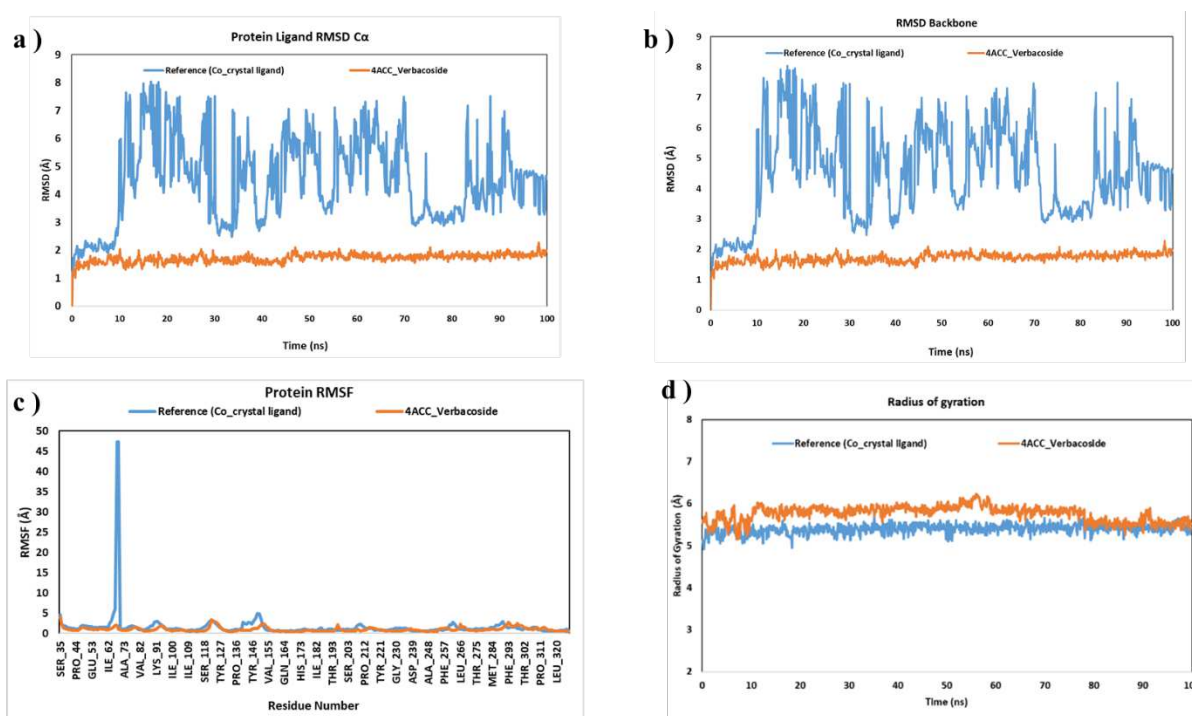


Figure 13: MD simulation results during 100ns run a) RMSD Calpha Reference Co-crystal Ligand and Verbacoside complex with 4ACC b) RMSD Backbone Reference Co-crystal Ligand and Verbacoside complex with 4ACC

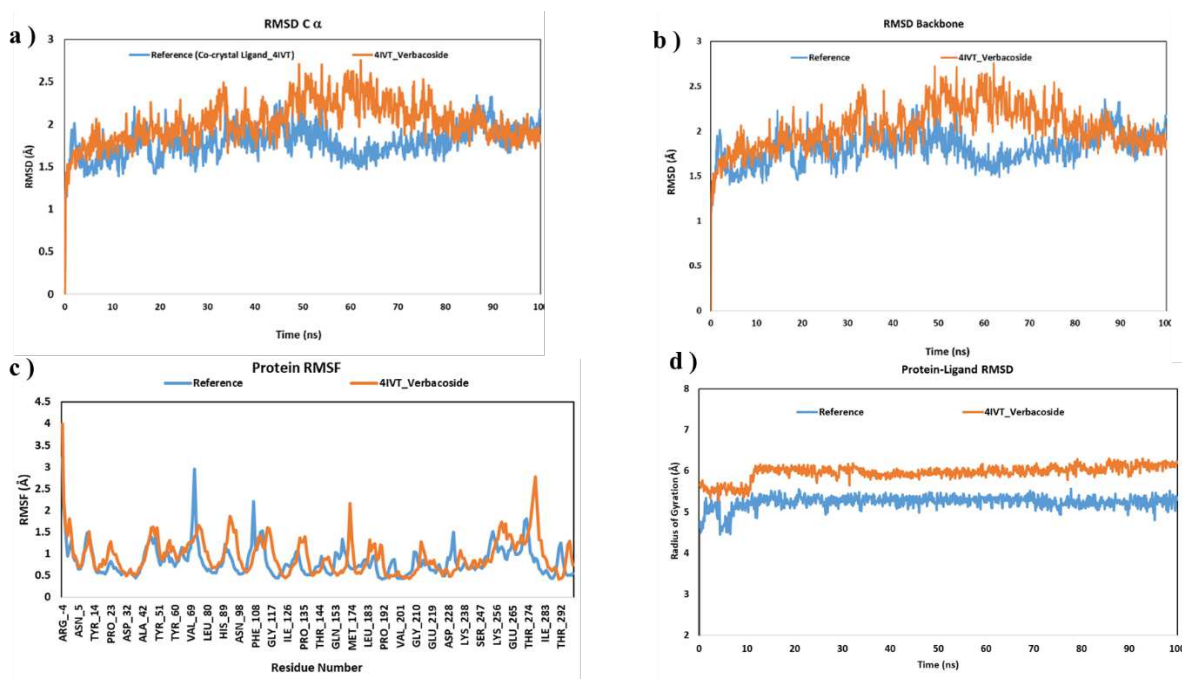


Figure 14: MD simulation results during 100ns run a) RMSD Calpha Reference Co-crystal Ligand and Verbacoside complex with 4IVT b) RMSD Backbone Reference Co-crystal Ligand and Verbacoside complex with 4IVT

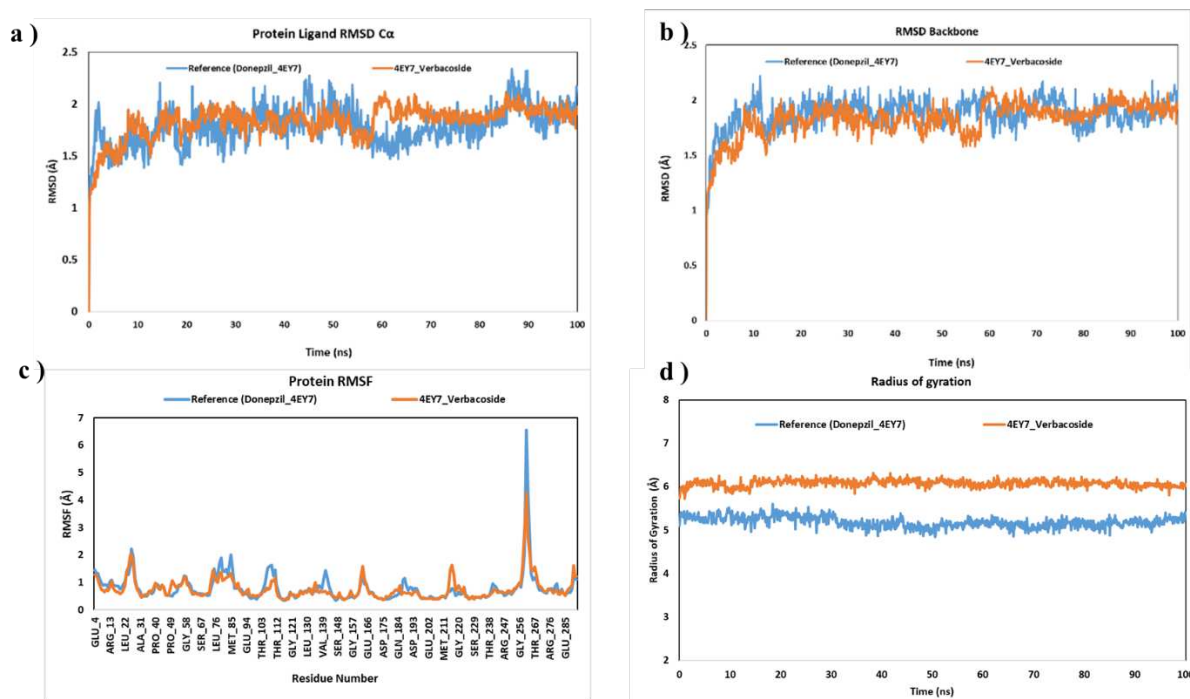


Figure 15: MD simulation results during 100ns run a) RMSD Calpha Reference Co-crystal Ligand and Verbacoside complex with 4EY7 b) RMSD Backbone Reference Co-crystal Ligand and Verbacoside complex with 4EY7.

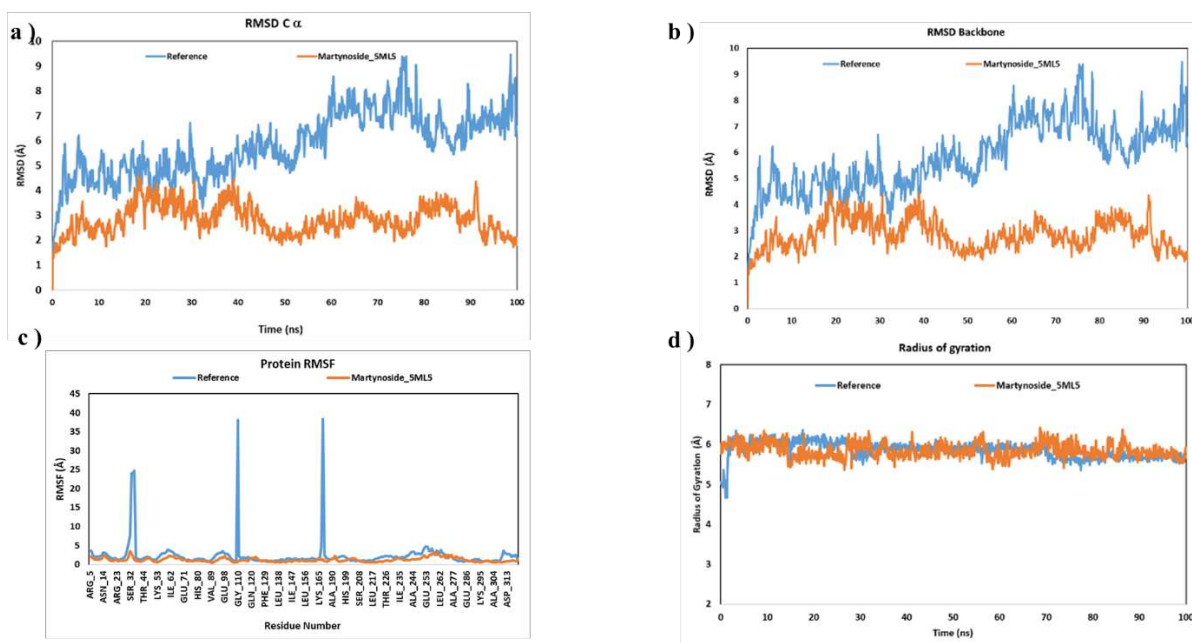


Figure 16: MD simulation results during 100ns run a) RMSD Calpha Reference Co-crystal Ligand and Marytoniside complex with 5ML5 b) RMSD Backbone Reference Co-crystal Ligand and Marytoniside complex with 5ML5.

3.10. MM-GBSA Analysis

MM-GBSA analysis was performed to estimate the binding free energy (ΔG_{bind}) of the ligand–protein complexes. For the AChE (4ACC) complex with verbascoside, the ΔG_{bind} was highly favorable at -165.99 kcal/mol, supported by strong contributions from van der Waals (-99.45 kcal/mol), Coulombic (-112.95 kcal/mol), and lipophilic (-80.05 kcal/mol) interactions. In the BACE1 (4IVT) complex, verbascoside exhibited a ΔG_{bind} of -73.58 kcal/mol, driven primarily by Coulombic (-59.20 kcal/mol) and van der Waals (-31.16 kcal/mol) interactions. For the MAPT (4EY7) complex, the ΔG_{bind} was -83.61 kcal/mol, with significant contributions from Coulombic (-65.26 kcal/mol) and van der Waals (-54.74 kcal/mol) energies, indicating a strong and stable binding. In contrast, for the GSK3 β (5ML5) complex with marytonoside, a positive ΔG_{bind} of +98.29 kcal/mol was observed, suggesting an energetically unfavorable binding under the simulated conditions. Overall, the MM-GBSA results confirm the strong binding affinity of verbascoside toward 5ML5, BACE1, and MAPT, consistent with the molecular docking and MD simulation findings that are summarized in Table 5, and Figure 17. [28].

Table 5: MMGBSA analysis score on various target of Alzheimer Disease

PDB ID	ΔG_{Bind}	ΔG_{Columb}	$\Delta G_{\text{Covalent}}$	ΔG_{HBond}	ΔG_{Lipo}	$\Delta G_{\text{Packing}}$	ΔG_{vdW}
	-165.991	-112.950	2.98094	-1.9802	-80.0539	-91.1928	

4ACC	-99.4458	-31.0933	1.9279	-2.0890	-38.3693	-3.08949	-55.8849
4IVT	-73.5815	-59.1968	3.2243	-6.3978	-24.6667	-0.2149	-31.1558
	-98.2237	-46.8345	1.1990	-2.1880	-36.9555	-0.5380	-53.6224
4EY7	-83.6056	-65.2634	2.1489	-0.8095	-48.4546	-1.4373	-54.7384
	-106.583	-44.1222	2.9836	-2.5854	-47.5542	-2.4525	-62.8130
5ML5	98.28604	-12.0828	2.5216	-0.6086	-49.3468	-0.2532	-48.2159
	-80.5359	-17.3954	0.5781	-0.9046	-38.5510	0	-46.4083

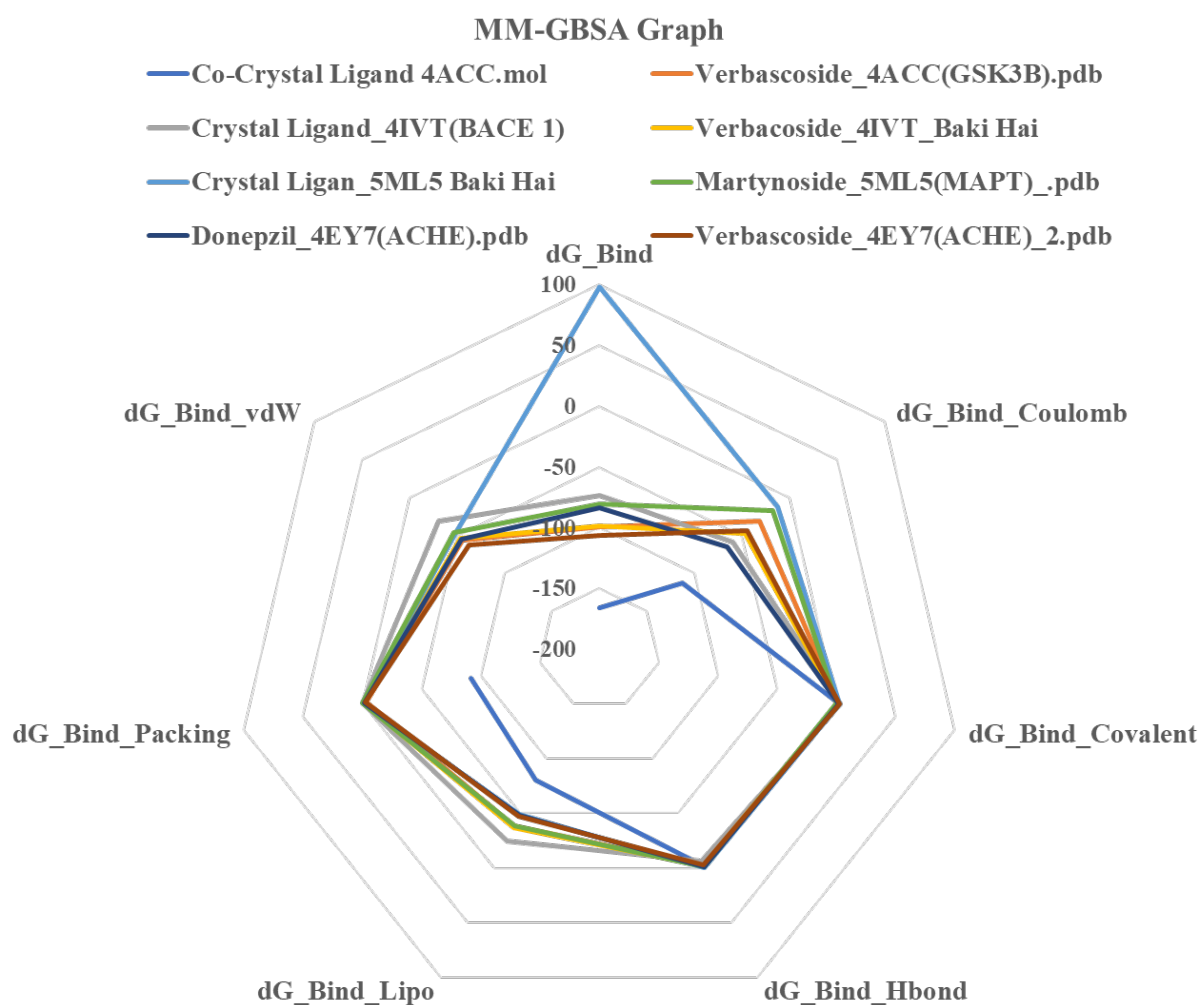


Figure 17: MM-GBSA Energy Decomposition for Multiple Alzheimer’s Disease Targets

3.11 Principal Component Analysis

PCA was conducted to evaluate the essential dynamics and conformational variability of the protein–ligand complexes. In the AChE (4ACC) system, both the co-crystal ligand and the verbascoside complex occupied similar conformational spaces, indicating comparable dynamic behavior with limited large-scale structural deviations shown in figure 18. For the BACE1 (4IVT) complex, verbascoside demonstrated stable motion patterns close to the reference

ligand, suggesting strong structural integrity during the simulation shown in figure 19. In the MAPT (4EY7) system, verbascoside exhibited dynamic movements similar to the co-crystal ligand, further confirming the stable nature of its interaction shown in figure 20. In the case of GSK3 β (5ML5), marytonoside also showed restricted conformational variability, indicating a relatively stable complex although less tightly clustered compared to the reference shown in figure 21. In the AChE (4ACC) system, the first two principal components (PC1 and PC2) captured approximately 28.8% of the total motion for the verbascoside complex, compared to 80% for the co-crystal ligand, indicating comparable conformational sampling. The distribution of conformations showed a tight cluster, with PC1 reflecting restricted flexibility. In the BACE1 (4IVT) complex, the verbascoside complex captured around 29.2% of the motion similar to the reference ligand i.e. 36.7%, suggesting stable dynamics. For the MAPT (4EY7) system, verbascoside covered 32.5% of the total variance, showing tight clustering compare with co_crystal ligand i.e. 53.3%, consistent with minimal conformational drift. In the GSK3 β (5ML5) complex, marytonoside showed slightly broader motions, capturing 31.1% of the variance, and co_crystal ligand has 32.7% suggesting moderate flexibility but overall structural stability. Overall, PCA results suggest that verbascoside and marytonoside maintain the structural stability of their respective targets, supporting the outcomes from RMSD and MM-GBSA analyses.

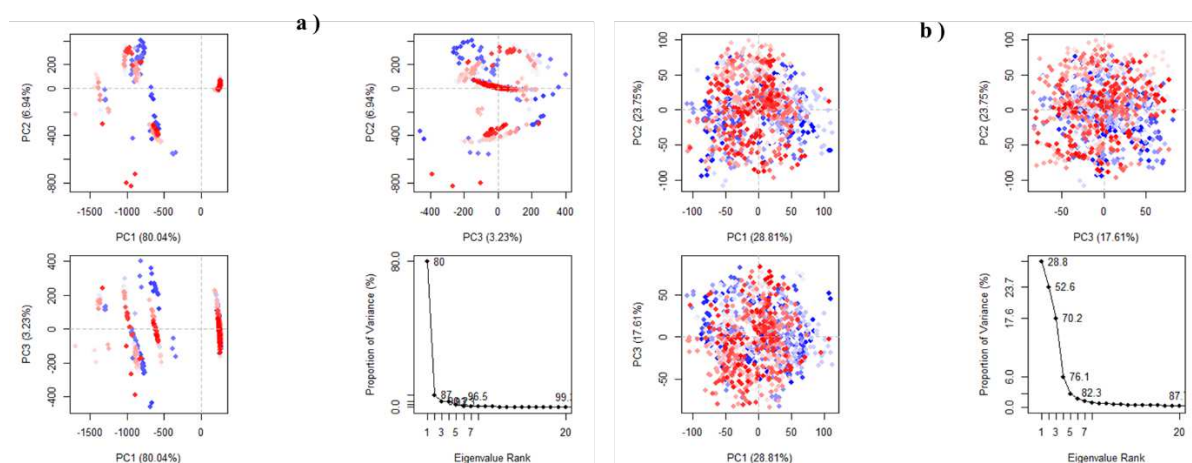


Figure 18: PCA of a) 4ACC_Co_Crystal ligand b) 4ACC_Verbacoside.

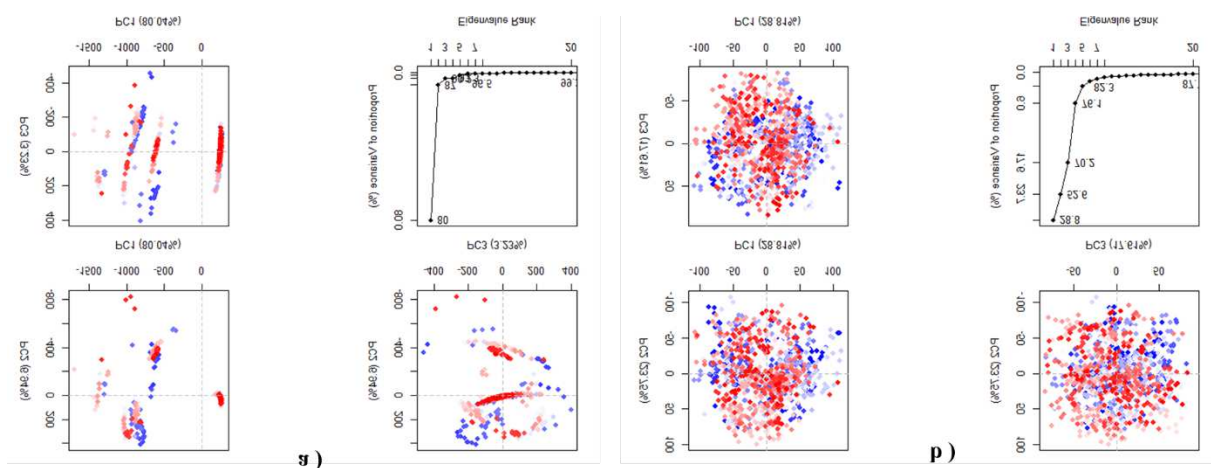


Figure 19: PCA of a) 4IVT_Co_Crystal ligand b) 4IVT_Verbacoside.

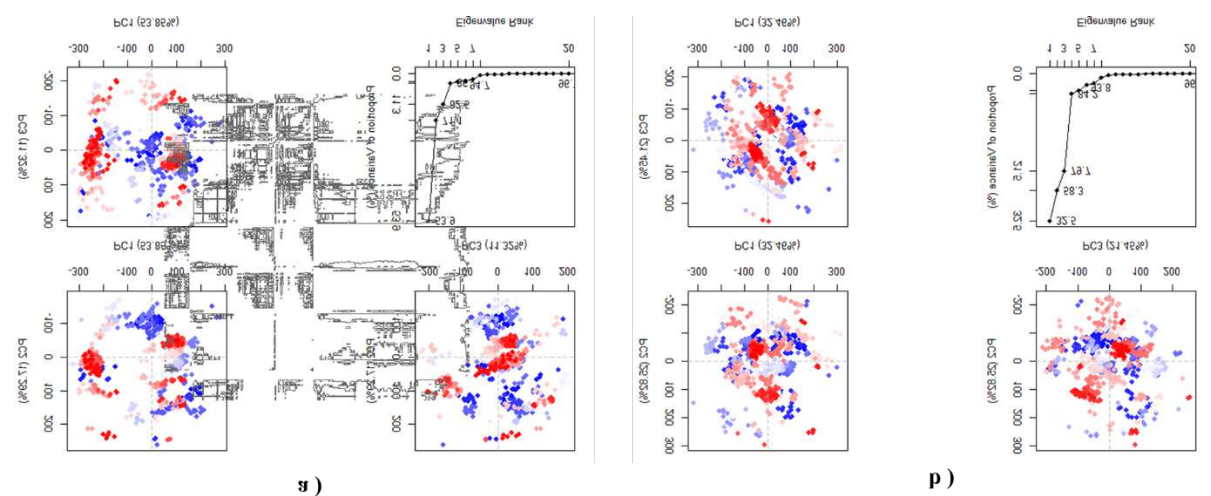


Figure 20: PCA of a) 4EY7_Co_Crystal ligand b) 4EY7_Verbacoside.

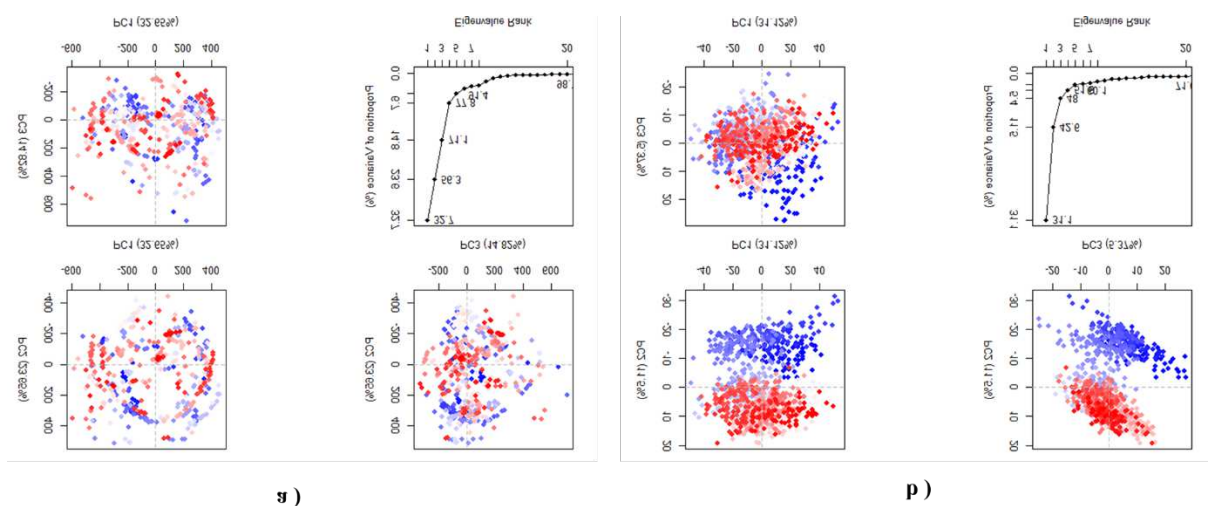


Figure 21: PCA of a) 4EY7_Co_Crystal ligand b) 4EY7_Verbacoside.

3.12. FEL Analysis

Free Energy Landscape (FEL) analysis was performed to explore the thermodynamic stability of the ligand–protein complexes. For the AChE (4ACC) complex, verbascoside exhibited a deep, well-defined global minimum similar to the co-crystal ligand, indicating a highly stable conformational state. In the BACE1 (4IVT) complex, verbascoside formed a relatively compact energy basin comparable to the reference ligand, reflecting stable binding. Similarly, the MAPT (4EY7) complex with verbascoside showed a prominent low-energy region, confirming minimal conformational fluctuations during simulation. For GSK3 β (5ML5), the marytonoside complex also displayed a stable free energy minimum, although slightly more scattered compared to the co-crystal ligand, suggesting moderate structural stability. Collectively, FEL analysis reinforces the notion that verbascoside and marytonoside form energetically favorable and stable complexes with their respective targets. For the AChE (4ACC) system, verbascoside exhibited a prominent low-energy basin with a minimum free energy of approximately -10 kcal/mol, closely matching the co-crystal ligand (around -12 kcal/mol), and confined within narrow RMSD ranges (C α RMSD between 1.5–2.5 Å and Radius of Gyration (Rg) between 19–21 Å) shown in figure 22. In the BACE1 (4IVT) complex, the global minimum was observed at around -8 kcal/mol for the verbascoside complex, compared to -9.5 kcal/mol for the reference ligand, indicating similar stability, with C α RMSD around 1.8–2.7 Å shown in figure 23. In the MAPT (4EY7) system, FEL of verbascoside showed a deep, stable energy minimum at approximately -9 kcal/mol, with RMSD values fluctuating between 1.7–2.5 Å shown in figure 24. For GSK3 β (5ML5), marytonoside displayed a shallower minimum at around -6 kcal/mol, with a slightly wider RMSD range (2.0–3.2 Å), suggesting moderate but acceptable conformational stability shown in figure 25. Overall, FEL analysis confirmed that verbascoside and marytonoside form thermodynamically favourable and structurally stable complexes with their targets.

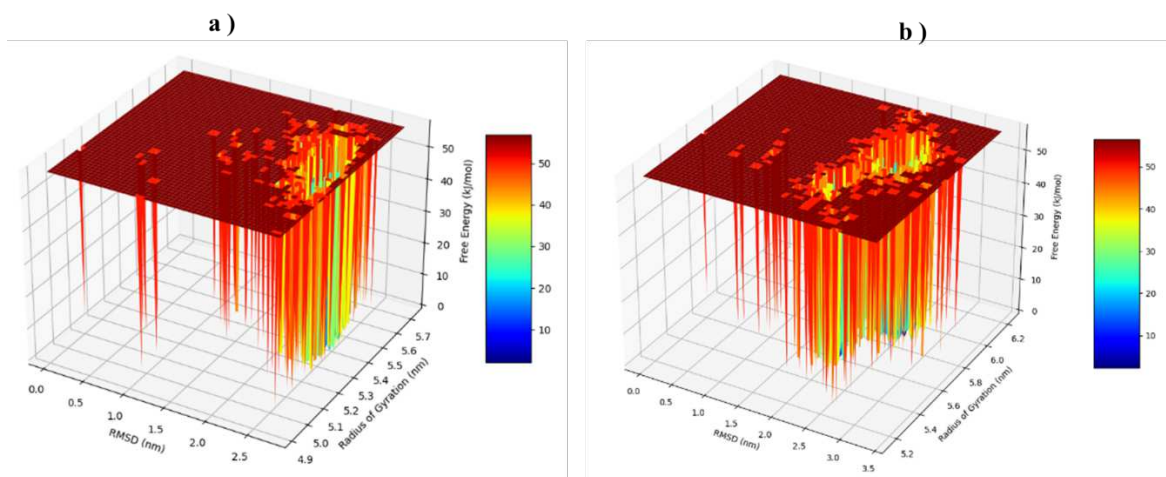


Figure 22: Free energy landscape of a) co_crystal ligand with 4ACC b) Verbacoside with 4ACC

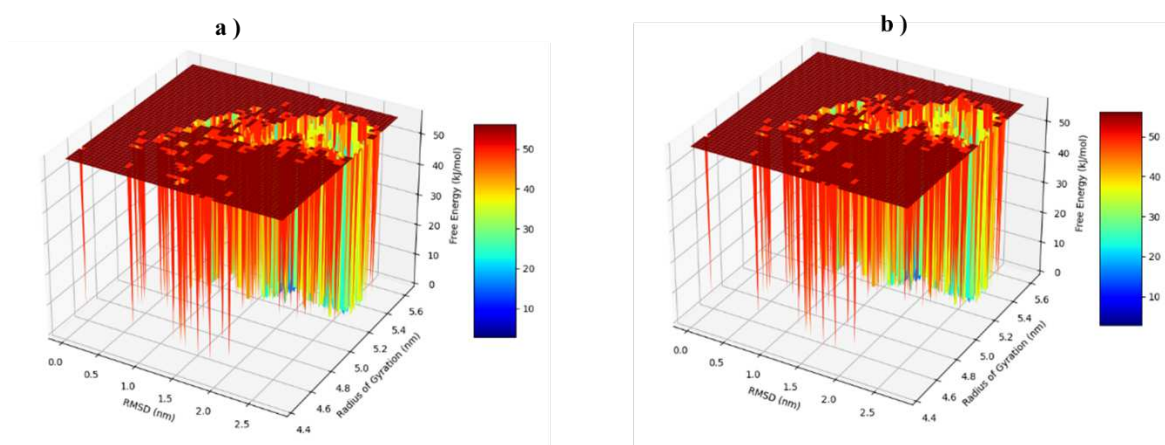


Figure 23: Free energy landscape of a) co_crystal ligand with 4IVT b) Verbacoside with 4IVT

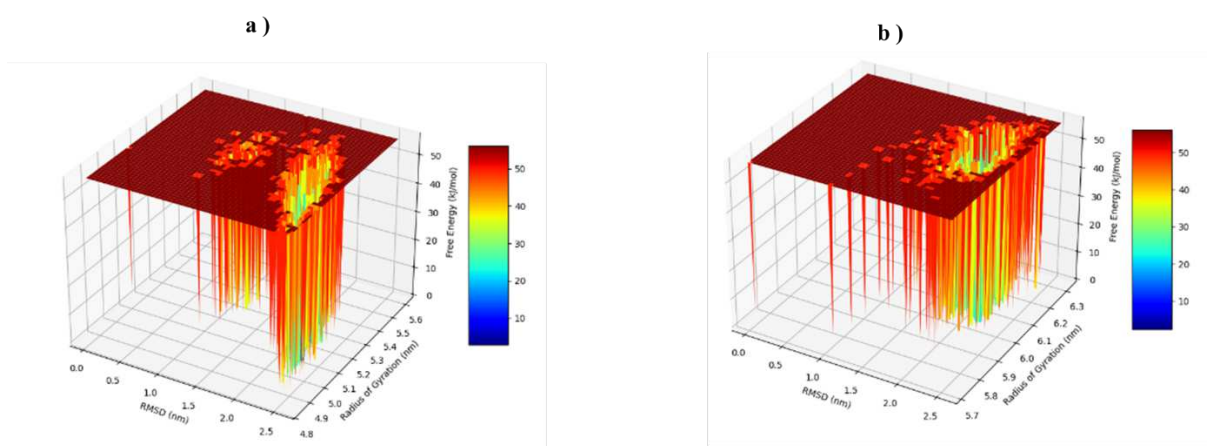


Figure 24: Free energy landscape of a) co_crystal ligand with 4EY7 b) Verbacoside with 4EY7

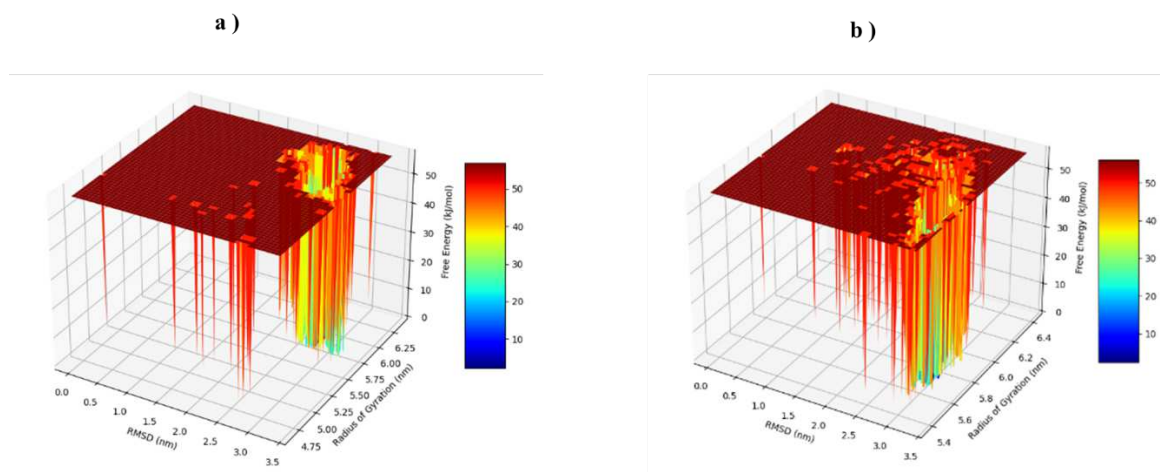


Figure 25: Free energy landscape of a) co_crystal ligand with 5ML5 b) Marytonisde with 5ML5

4. Discussion

AD is one of the most common forms of dementia [67]. As the world's population ages and the prevalence of AD continues to rise, AD has become an urgent health problem. The main pathological feature of AD is the deposition of extracellular A β and intracellular NFTs that are composed of hyperphosphorylated tau proteins, leading to neuronal damage and loss [68]. At present, some clinical drugs can only relieve symptoms but cannot stop the process of neurodegeneration, indicating there is still a lack of effective and thorough treatments for this disease [69]. Therefore, new drug research and development or the development of new natural drugs that can treat AD is of great significance.

Given the polypharmacological nature of medicinal plants, GA presents an opportunity to address the multifactorial nature of AD by targeting multiple signaling pathways rather than a single molecular entity. The present study provides a comprehensive network pharmacology and molecular modeling analysis of GA in the context of AD. By integrating bioinformatics-driven target prediction, PPI network construction, pathway enrichment, molecular docking, MD simulations, MM-GBSA, PCA, FEL this investigation highlights the multi-target potential of GAPC in modulating key AD-related pathological mechanisms. 105 potential targets of 16 GAPC were identified using network pharmacology approaches. A key strategy in this study was the selective exclusion (from molecular docking and subsequent experiments) of extensively reported GAPC such as umbelliferone[70], luteolin-7-glucuronide[71], kaempferol [72], quercetin [73], apigenin 7-glucuronide, apigenin [74], luteolin [75], campesterol, stigmasterol, beta-sitosterol, and stigmasterol [76]. Additionally, 7-Geranyloxy-5-methoxycoumarin was also deleted from the network as it was associated with single target (which was also not involved with other GAPC). These plant metabolites have been widely

studied for their neuroprotective effects, including cholinesterase inhibition, antioxidant activity, and modulation of inflammatory mediators. While previous research has demonstrated the potential of these phytoconstituents in AD, the novelty of this study lies in its focus on lesser-explored compounds (verbascoside, martynoside, quercetagenin, and tyrosol), to uncover new therapeutic candidates via exploring their interaction with multiple receptors involved in AD (BACE1, MAPT, AChE and GSK3B).

GA could exert its anti-AD effect through multi-targets and multi-pathways. Core targets play a crucial role in the entire network and may play crucial role in the management of AD. In our study, top ten core targets were AKT1, EGFR, ESR1, SRC, PTGS2, GSK3B, MMP9, PARP1, KDR, and ABCB1. These targets play crucial roles in the pathophysiology of AD by influencing key processes such as A β accumulation, tau hyperphosphorylation, neuroinflammation, oxidative stress, vascular dysfunction, and synaptic plasticity. AKT1 supports neuronal survival, while EGFR and PTGS2 drive neuroinflammation. Estrogenic signalling of ESR modulates cognitive function, whereas SRC impacts tau pathology. GSK3B is a major player in tau hyperphosphorylation, and MMP9 contributes to blood-brain barrier disruption. PARP1 dysregulation leads to neuronal damage, KDR affects neurovascular integrity, and ABCB1 dysfunction impairs amyloid clearance. While the top ten identified targets exhibit therapeutic potential, it is crucial to recognize that AD is a multifactorial disorder requiring a comprehensive approach that addresses multiple pathological pathways. Some of these targets may not warrant further computational investigation, as their roles appear to be more peripheral or less directly implicated in the core mechanisms underlying AD pathogenesis compared to key targets such as BACE1, MAPT, and AChE. Consequently, for molecular modeling studies, four primary receptors, BACE1, MAPT, GSK3B, and AChE were selected. Notably, GSK3B was also identified within cluster one. Additionally, AChE, BACE1, and MAPT were also identified in cluster two; further supporting their major relevance in AD pathology. Targeting these proteins may offer a multi-faceted therapeutic approach to counteract progression of AD.

GO enrichment analysis revealed that the identified AD-related targets were significantly associated with core BP, including cellular and metabolic processes, biological regulation, and response to stimulus. These processes are crucial in neurodegeneration, as impaired metabolic regulation and dysfunctional cellular responses contribute to AD pathogenesis [77]. The significant enrichment of cellular response to stimulus aligns with growing evidence that neuroinflammation and oxidative stress play pivotal roles in neuronal dysfunction. Similarly, primary metabolic processes were represented, indicating the

involvement of GAPC in pathways that regulate neuronal energy homeostasis and neurotransmitter metabolism. This is particularly relevant; as metabolic dysregulation is increasingly recognized as a key driver of AD progression[78]. These findings implicated the involvement of multiple targets and various BP in multiple synergistic effects of GAPC against AD.

CC analysis further emphasized the relevance of intracellular structures, such as organelles, membrane-bounded organelles, and intracellular anatomical structures, highlighting the role of GAPC in modulating molecular interactions within neuronal compartments. The observed enrichment of nuclear and cytoplasmic components suggests that these phytochemicals may influence gene transcription, protein synthesis, and intracellular signaling cascades. Given that many neuroprotective phytoconstituents exert their effects through epigenetic and transcriptional regulation, these findings provide a mechanistic basis for effects of GA against AD.

MF analysis revealed strong associations with binding interactions, including ion binding, catalytic activity, and protein binding. The enrichment of ion-binding functions suggests that GA phytochemicals may influence metal ion homeostasis, a critical factor in AD pathology given the involvement of iron, copper, and zinc dysregulation in A β aggregation.

KEGG pathway enrichment further elucidated the molecular mechanisms through which GAPC exerts their neuroprotective effects. The identification of pathways such as PI3K-Akt, MAPK, FoxO, Ras, and Rap1 signaling aligns with existing research demonstrating their involvement in neuronal survival, synaptic plasticity, and neuroinflammation. Notably, the strong enrichment of the PI3K-Akt pathway suggests that GAPC may promote neuronal resilience by enhancing pro-survival signaling and reducing apoptosis. Similarly, modulation of the MAPK pathway indicates potential anti-inflammatory and antioxidant effects, as this pathway plays a crucial role in regulating microglial activation and oxidative stress responses in AD.

In this study, a network pharmacology-driven approach highlighted several key targets implicated in AD pathogenesis, including BACE1, MAPT, AChE, and GSK3 β . These targets, due to their biological relevance and centrality in the PPI network and enriched pathways (e.g., PI3K-Akt and MAPK), were prioritized for in-depth computational modeling to assess the multi-target potential of selected GAPC.

Given the complex, multifactorial nature of AD, the investigation focused on lesser-explored, yet bioactive GA-derived compounds, verbascoside, martynoside, quercetagenin, and tyrosol. Among these, verbascoside and martynoside consistently demonstrated strong binding

affinity and dynamic stability across multiple AD targets, suggesting their potential as multitarget-directed ligands. Their interactions with AChE and MAPT are particularly noteworthy, as these targets play central roles in the cholinergic and tau-related pathology of AD, respectively.

MD simulations supported these findings by indicating stable protein–ligand complexes, especially in the case of verbascoside with AChE, BACE1, and MAPT. Binding free energy calculations using MM-GBSA further reinforced the stability and favorability of these interactions, with van der Waals and electrostatic forces contributing significantly to complex formation. While martynoside showed stable interactions, particularly with MAPT and GSK3 β , its binding with GSK3 β appeared thermodynamically weaker, pointing to the importance of assessing both structural and energetic parameters in multitarget-directed ligands evaluations.

Additionally, PCA and FEL analysis provided insight into the global conformational dynamics and energetic stability of the protein–ligand systems. Verbascoside exhibited reduced conformational fluctuations and favorable low-energy states across multiple targets, suggesting its capacity to stabilize target proteins and potentially modulate their pathological functions. In contrast, tyrosol showed limited interaction potential and dynamic stability, indicating a lower likelihood of significant therapeutic benefit.

Overall, the integrative computational strategy employed in this study, from network pharmacology to advanced simulation techniques, highlights verbascoside as a promising multitarget compound capable of modulating key AD targets. Martynoside also shows favorable properties, albeit to a slightly lesser extent. These findings highlight the potential of selected GAPC in AD therapeutics, particularly within a polypharmacological framework aimed at addressing the multifaceted nature of the disease.

5. Conclusion

This study presents a systematic network pharmacology and molecular modeling approach to investigate the multi-target therapeutic potential of GA against AD. While ten core targets were identified, a strategic focus was placed on four key targets (BACE1, MAPT, AChE and GSK3B) due to their central role in AD pathogenesis. These targets are implicated in critical pathological mechanisms such as amyloid aggregation, tau hyperphosphorylation, cholinergic dysfunction, and neuroinflammation, making them prime candidates for computational studies. Notably, instead of extensively studied GAPC, this study prioritized lesser-explored bioactive compounds from GA for molecular modelling to identify novel therapeutic candidates. Together, these integrated findings suggest that verbascoside is the most potent multi-target

inhibitor, capable of addressing multiple pathological aspects of AD, including cholinergic dysfunction (via AChE inhibition), amyloidogenesis (via BACE1), tau aggregation (MAPT), and kinase dysregulation (GSK3 β). Martynoside also demonstrated strong multitarget potential, particularly against MAPT and AChE. These two phytoconstituents warrant further in vitro and in vivo validation for potential development as natural, multitargeted therapeutics for AD. Overall, this study confirms GA as a promising multi-target therapeutic candidate for AD. By identifying and validating lesser-explored bioactive compounds, it offers new directions for phytochemical-based drug discovery. However, given the complexity of AD, further in vitro and in vivo studies are necessary to confirm these computational findings and assess their clinical relevance.

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Declarations**Competing interests**

The authors declare no competing interests

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