https://doi.org/10.48047/AFJBS.6.14.2024.12112-12142

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

In Silico Evaluation of *Bacopa monnieri's* Saponins as Promising Candidates for Cognitive Impairment Therapeutics

Shamshad Ather[1], Radhika Sharma[1], Yogesh Patil[1], Himanshu Gupta[1], Mansee Thakur*[1]

[1]Department of Medical Biotechnology, MGMSBS, MGMIHS, Kamothe, Navi Mumbai, Maharashtra, India

Volume 6, Issue 14, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.14.2024.12112-12142

Abstract

Ayurvedic plants hold promise for drug discovery as they are rich in bioactive phytochemicals. *Bacopa monnieri* (BM) is one of these plants that has been used to treat cognitive impairments (CI). Therefore, this study evaluates the pharmacological impact of the saponins found in BM on CI using *in-silico* approach. 32 saponins from BM were examined for ADMET. Their targets were predicted, out of 217 targets 13 were found to be involved in CI. These were further analyzed using pathway enrichment, docking and simulation tools. It was observed that some of the saponins had good affinity for SCN2A and DNMT1. Further Molecular Dynamics simulation for 100 ns shows the stability of complex. These two proteins are known to play crucial role in development of CI. The findings represent a significant step towards understanding the medicinal properties of BM which may be the potential drug candidates for the treatment of CI.

Keywords: *Bacopa monnieri*, Saponins, Phytochemicals, Bioinformatics, ADMET, Network pharmacology, Docking, Simulation

Introduction

Ayurveda is a system of profound paradigms for the maintenance of well-being (Shankar, 2023). This traditional system is grounded in herbal remedies, emphasizing a holistic approach to health and wellness (Jaiswal et al., 2016). Ayurvedic formulations and their constituent natural ingredients hold promise as a rich source of bioactive compounds for further scientific investigation and potential drug discovery (Zhang et al., 2023). Several herbs like Brahmi, Ashwagandha, Sage, and Turmeric have been traditionally utilised as brain or nerve tonics in Indian folk medicine (Malík et al., 2023).

Bacopa monnieri (BM), a nootropic herb, commonly known as Brahmi in India has been utilized for millennia in Ayurveda. It is a small perennial herbaceous plant, belonging to the family Scrophulariaceae, used in traditional medicine to cure a variety of nerve illnesses, provide digestive aid, enhance learning, memory, and focus, as well as to relieve anxiety in patients and treat skin conditions (Malík et al., 2023, Delfan et al., 2024). The *Bacopa* plant enhances brain function by restoring synaptic activity, neuronal synthesizing, and healing injured neurons (Jeyasri et al., 2020). BM was and is still regarded as an effective Indian herb for treating brain illnesses, age-related mental decline, and enhancing cognitive abilities (Banerjee et al., 2021). Multiple mechanisms of action have been suggested to explain its cognitive effects, such as inhibition of acetylcholinesterase (AChE), reduction of β-amyloid, activation of choline acetyltransferase, and increased cerebral blood flow (Banerjee et al., 2021, Singh et al., 2021).

The plant contains a diverse range of pharmacologically active compounds, contributing to its multifaceted medicinal properties (Jeyasri et al., 2020). The Brahmi extract has been identified to contain compounds categorized as triterpenoids saponins, alkaloids, glycosides, and alcohols (Dubey et al., 2019). The primary active constituents are dammarane-type triterpenoid

saponins, featuring jujubogenin and pseudojujubogenin as the aglycones (Fatima et al., 2022). These saponins encompass various subtypes known as bacosides, bacopasides, and bacopa saponins. Among them, Bacoside-A is identified as the major active component (Fatima et al., 2022).

Numerous studies using in vitro, in vivo and clinical research over the last two decades have been conducted on BM extract and its separated components to support the traditional claims on the effectiveness of *Bacopa* plant. (Singh **B** et al., 2021; Banerjee et al., 2021) Despite its potential, the specific components and mechanisms behind BM's activity remain unclear. Noticing this the research aims to identify the phytochemicals in BM that may influence proteins related to cognitive impairment. Through screening these phytochemicals and their protein targets, this study utilizes computational methods such as molecular docking and simulations to explore the various proteins BM might influence. This investigation also includes network pharmacology to investigate the potential of BM phytochemicals to modulate the activity of neurotrophins, molecules crucial for brain function, concerned with cognitive decline.

1. Methodology

2.1 Ligand Library Preparation

A comprehensive exploration of *Bacopa monnieri* (BM) was undertaken through an detailed internet search, utilizing diverse databases such as Google Scholar (https://scholar.google.com/) , PubMed (https://pubmed.ncbi.nlm.nih.gov/) , Research Gate (https://www.researchgate.net/) , Science Direct (https://www.sciencedirect.com/) , and various journals. The identified phytochemicals were cataloged, and their canonical Simplified Molecular Input Line Entry System (SMILES) representations and 2D structures in sdf format

were retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) Compound database. To enhance the obtained 2D structures, the Avogadro software (Hanwell et al., 2012) was employed for the conversion into 3D structures. Ligand structures were further prepared by adding nonpolar hydrogens, Gasteiger changes, and rotatable bonds and converted in .pdbqt format using AutoDock Tools.

2.2 Target Prediction and their Comparative Modelling

The SMILES data sourced from PubChem served as the essential input for target prediction through two distinct servers, namely Super-PRED (https://prediction.charite.de/) and Way2Drug (https://www.way2drug.com/passonline/). The outputs generated were subjected to a thorough sorting and filtering process based on prediction scores and model accuracy criteria. Subsequently, the compiled target list was cross-referenced with a catalog of genes associated with cognitive impairment, obtained from the Human Phenotype Ontology (HPO) database (https://hpo.jax.org/app/). Genes common to both datasets were selected for further detailed examination.

The common genes/Target proteins were then modelled due to the presence of missing residues in the PDB protein obtained. The software used to do so was Swiss Modeller. For this the amino acid sequence was obtained from UniProt and to achieve model accuracy the structures with more than 75% coverage were taken into account (Singh A et al., 2020). Targets were further prepared by removing all water molecules and heteroatoms, accompanied by minimizing energy and conversion to .pdb format with the help of the BIOVIA Discovery Studio visualizer. Polar hydrogen atoms and Kollman charges were added to the protein structures to prepare them for molecular docking (Joshi et al., 2022). After that, the protein's pdb structure was converted to pdbqt, and grid preparation was done using AutoDock Tools version.

2.3 Network Pharmacology

The pathway analysis and evaluation of the identified targets were conducted systematically, involving a meticulous examination of interconnected signalling pathways and molecular interactions of the targets. The primary objective was to gain a comprehensive understanding of how the chemicals identified in the study might modulate various pathways, thereby illuminating their effects on cognitive function and central nervous system disorders. This study employed the Cytoscape (Shannon et al., 2003) app, ClueGo (Bindea et al., 2009), SR Plot, GO Pathway and KEGG Pathway.

2.4 ADMET Studies

Drug likeness refers to a compound's potential to function as a drug, which is evaluated through various studies such as adsorption, digestion, metabolism, excretion, and toxicity. These studies are crucial in assessing a drug's safety and effectiveness during early development. To pass ADMET analysis, molecules must meet specific criteria, with lipophilicity (LogP) being a key consideration. LogP values influence the absorption rate of drug molecules. For anti-neurodegenerative drugs, an effective LogP range is typically 1-2. Factors such as the Blood Brain Barrier (BBB), Human Intestinal Absorption (HIA), and Caco-2 values are important in drug development, particularly for neurodegenerative drugs. Human Intestinal Absorption (HIA) denotes a drug's absorption extent in the human system, while Caco-2 values indicate oral drug ingestion. Toxicity testing, is often conducted using the Ames test. The Ames test is a biological examination used to assess the mutagenic properties of a substance, which is critical in drug synthesis to ensure safety (Pajouhesh et al., 2005). Through AI Drug Lab (https://ai-druglab.smu.edu/admet) server analysis, drug-likeness features including lipophilicity, solubility, and drug-likeness score were determined based on uploaded

phytochemical structures.

2.5 Molecular Docking and Dynamic Simulations

AutoDock Vina was utilized for the docking of target proteins and ligands. Using AutoDock Vina loaded in the Dockey (Du L et al., 2023) BM's phytochemicals were docked to each receptor using grid coordinates and grid boxes of specific sizes. For every ligand and protein, the optimal binding position was determined at 0 RMSD (Root Mean Square Deviation). The binding affinity was used to rank the results. Hydrophobic and hydrogen-bond interactions, ligand efficacy matrixes, inhibition constant as well as bonding distances have also been explored to investigate protein-ligand docking poses and promising confirmations were chosen.

Following that, to further guarantee flexibility and stability molecular dynamics simulations were executed. The top five docked complexes with the best binding free energies and maximum number of hydrogen bonds were chosen. Desmond Schrodinger 2023.2 (Bowers et al., 2006) was used to do an MD simulation for 100 ns. Hydrogens were added to protein-ligand complexes to complete residues or replace missing side chains to prepare them for simulation. It involved manually adjusting the tautomeric and protonation states of the variable residues, which was followed by the creation of a solvated system for simulation. The complexes were solvated using the TIP3P water model, and equilibrated under 0.1 ns isothermal-isobaric (NPT) ensembles. Energy minimization was carried out after neutralization. For the simulations, OPLS_2005 force field parameters were utilized. The system's temperature and pressure were maintained at 310 K and 1 atm, respectively. At 4.0 ps intervals, trajectories were sampled to obtain detailed data. The Desmond package's unique analytical tool was used to look into how saponins interacted with its molecular targets. Two

essential indicators were used to assess complex stability: Root Mean Square Deviation (RMSD), which measures the complex's structural deviations from a reference state.

2. Results

Upon extensive research from diverse databases Direct it was found that 108 phytochemicals were listed up to date belonging to different groups such as alkaloids, saponins, ethers, etc that are present in *B.monnieri*. Following these compounds and their previous studies it was found that a specific group of phytochemicals called 'Saponis' are majorly involved in cognition and CNS related disorders. **Table 1** shows the 32 Saponins found in *Bracopa monnieri.*

3.1 Saponin's Targets involved in Cognitive Impairment and their Homology Modeling

To explore potential protein targets for Saponins, our investigation employed two distinct target prediction tools: way2drug and Super-PRED. Results reveled that saponins are involved in function of 217 proteins in humans. To obtain proteins involved in cognition we curated a comprehensive dataset consisting of 958 proteins known to be associated with cognitive impairment, sourced from the Human Phenotype Ontology database. This dataset served as a reference point to assess the potential relevance of Saponins target proteins in the context of cognitive function.

The results from our target prediction analysis with the cognitive impairment protein dataset were compared utilizing Venny 2.0 for Venn diagram analysis. This analytical approach enabled us to identify 13 target proteins that were found to be common between Saponins target proteins and the proteins involved in cognitive impairment as shown in **Figure 1**. Whereas **Table 2** shows the list of 13 targets finalized and their full forms reflecting their functions.

To enhance our understanding of the molecular interactions involved and to fill missing residue, we undertook modelling of all 13 target proteins. Among these targets, 7 proteins—CCR1, GBA1, MTOR, SCN2A, SCN3A, DNMT1, and TLR4—were found to have a minimum coverage of 75% of amino acid residues [19]. These structures were prioritized for in-depth investigations involving Docking and Molecular Dynamics simulations. The remaining targets— EP300, THRB, KIF11, HDAC4, TNIK, and BCR—were exclusively utilized for network analysis.

3.2 Network Ethnopharmacology and Saponin Target Dialogue

To explore the multifaceted roles of 13 potential cognitive impairment (CI) targets of Saponins found in *Bacopa monnieri*, KEGG pathway enrichment and Gene Ontology (GO) analysis on these candidate targets were carried out. Our analysis revealed significant enrichment of these targets in 1053 biological processes (BP), 51 cellular components (CC), and 58 molecular functions (MF). It uncovered a diverse array of biological processes associated with saponins, of which the top 3 were regulation of myeloid cell differentiation (GO:0045637), regulation of osteoclast differentiation (GO:0045670), and osteoclast differentiation (GO:0030316). Voltage-gated sodium channel complex (GO:0001518), sodium channel complex (GO:0034706), and cation channel complex (GO:0034703) were top three cellular components which shed light on the subcellular localization of saponin's activities. Saponins were also found to participate in a wide spectrum of molecular functions, encompassing voltage-gated sodium channel activity (GO:0005248), sodium channel activity (GO:0005272), and sodium ion transmembrane transporter activity (GO:0015081) mainly involving proteins SCN2A and SCN3A. Utilizing KEGG pathway analysis, we investigated the potential impact of saponins on cellular pathways. A total of 93 pathways were identified, suggesting a multifaceted influence of various saponins on diverse cellular processes. Among the identified pathways,

the top three pathways with the most significant impact based on enrichment scores were HIF-1 signaling pathway (hsa04066 Kaposi sarcoma-associated herpesvirus infection (hsa05167) and MicroRNAs in cancer (hsa05206).

The depiction in **Figure 3** visually highlights the significant association between the 13 common targets of saponins, represented in purple, and major cognitive impairment illnesses, outlined in green, emphasizing the intricate relationships between these targets and cognitive impairment illnesses. This network analysis serves as a crucial tool in unravelling the complex interactions between compounds and their target proteins. The visualization and analysis of these intricate relationships, with targets in purple and pathways in green, offer valuable insights into the complex landscape of cognitive impairment. This comprehensive approach contributes significantly to our understanding of the molecular mechanisms underlying cognitive impairment. The precision of this molecular network analysis, where connecting edges represent specific proteins, opens new opportunities for therapeutic strategies in cognitive impairment. Validation analyses further support the robustness of the identified common targets, adding depth to our understanding of the complex landscape associated with cognitive impairment and providing a solid foundation for future research and therapeutic development efforts.

3.3 ADMET Analysis

ADMET analysis of the saponins present in BM (**Table 3**) revealed that the lipofilicity value of all the 28 phytochemicals falls under desired range of 1-2 for anti-neurodegenerative drugs. This indicates that all the saponins present in BM shows high absorption. The percentage blood-brain barrier value of all the saponins falls under the category of CNS active compound. Suggesting a good absorption all the 28 saponins show modest intestinal absorption defined between these two values (30% < HIA < 79%) (Perez et al., 2004) with Bacopaside A3,

Bacoside A1 and Bacopasaponin G showing highest HIA. Representing ingestion of orally administered drug, all the Caco-2 values represented a reasonable permeability. Ames analysis for toxicity of the saponins reviled that all the phytochemicals belongs to moderate non-mutagenic category.

3.4 Molecular Docking and Dynamic Simulation

In our study, we utilized 28 saponins as ligands in Molecular Docking Analysis, employing the Dockey software. Our primary objective was to assess the binding affinity of these ligands with 7 common receptors, which were modelled using the SWISS-Model. All the 210 docked results were studied. This evaluation involved a comprehensive analysis of hydrophobic and hydrogen-bond interactions, ligand efficacy matrices, inhibition constants, and bonding distances. To ensure the reliability and robustness of our findings, we implemented stringent criteria. Specifically, we selected complexes with binding affinities of at least -4 kcal/mol. additionally, we considered ligand efficiency (LLE) and solvent-inaccessible ligand efficiency (SILE), setting thresholds of at least 4.43 and 2.5, respectively (Hopkins et al., 2014; Kenny et al., 2019). The results of our analysis unveiled notable binding affinities and ligand efficiency values across various saponins and their respective targets. Top 10 docking results are shown in **Table 4** depicting receptor and ligand binding affinity, LLE and SILE. All the docking results are provided in supplementary information.

The results as shown in **Figure 4** indicate a strong interaction between saponins and their specified targets, highlighting its potential significance in pharmacology. As mentioned in **Table 5,** Bacoside A1 achieved the highest docking score, with the value of -11.33 kcal/mol establishing hydrogen bonds with amino acid residues Lysine (Lys1422), Glutamine (Gly383) and Tyrosine (Tyr1771) of receptor SCN2A. A salt bridge is observed between SCN2A and Bacoside A1 at residue Lysine (Lys1422) along with two hydrophobic interactions at Leucine

(Leu983) and Isoleucine (Ile146). Whereas, DNMT1, acting as a receptor engages in interaction with ligands Bacoside A2 (Binding affinity -9.53 kcal/mol), Bacopaside N1 (Binding affinity -10.34 kcal/mol) and Bacopasaponin E (Binding affinity -9.07 kcal/mol) forming eight, eight and twelve hydrogen bonds respectively along with three, three and two hydrophobic bonds. SCN2A is seen to form hydrogen bonds with Bacoside A2 at amino acid residue Lysine (Lys1422), Serine (Ser1713) and Glutamine (Gln383) with a binding affinity of -10.34 kcal/mol. Two Hydrophobic interactions are also seen to be formed between Phenylalanine (Phe378) and Isoleucine (Ile420).

In this study, we also conducted MD simulations over a 100 ns timeframe to assess the stability and dynamic behavior of five ligand-protein complexes. The root mean square deviation (RMSD) and graphs were generated for these complexes to compare their positions with the initial positions. RMSD analysis offered insights into structural deviation, conformational stability, and convergence during the simulation period. In a well-equilibrated system, the RMSD towards the end of MD simulations is expected to be within 1–3 Å for a globular protein. The time-dependent RMSD graphs were plotted for SCN2A-Bacoside A1, DNMT1A-Bacoside A2, SCN2A-Bacoside A2, DNMT1-Bacopaside N1 and DNMT1-Bacopaside E complexes. Examination of the RMSD plots for ligands and receptors revealed multiple binding orientations for the ligands. The plot illustrates the RMSD values for the protein (left Y-axis) and ligand (right Y-axis). Lig fit Prot and Lig fit Lig indicate the RMSD of a ligand when the protein-ligand complex is first aligned on the protein backbone of the reference and its reference conformation, respectively. Bacoside A1 docked with SCN2A receptor gets stabilized after 15-20 ns and does not experience any changes thereafter. In the case of docked Bacoside A2 and DNMT1 the Root Mean Square Deviation (RMSD) stabilized at around 40 nanoseconds (ns), indicating that the complex maintains stability and does not undergo substantial conformational alterations in the protein structure throughout the simulation period.

When Bacoside A2 was docked with the SCN2A receptor, the complex became stabilized within 15 to 20 nanoseconds (ns) and remained unchanged thereafter. The complex between DNMT1 and Bacopaside N1 is seen to get stabilized for a period of 20ns between 20ns to 50ns before experiencing a shift at 60ns. Bacopasaponin E and DNMT1 complex seem to attain stability between 20ns and 80ns with some conformational changes before and after that.

The stability of protein-ligand complexes during simulations relies on factors like intermolecular hydrogen bonds, which were assessed through the analysis of simulation trajectories. Apart from hydrogen bonds, hydrophobic and ionic interactions are also crucial in governing protein-ligand interactions. **Figure 5** provides a detailed schematic representation of how ligand atoms interact with specific protein residues.

3. Discussion

As the population ages, neurodevelopmental disorders and cognitive decline pose significant challenges. Neurocognition and attention are crucial for daily life; however, many synthetic drugs used to treat these issues come with side effects due to their chemical composition (Malik et al., 2022). In recent years, both in industrialized nations and the west, interest in using herbal products has rapidly increased. Among these herbs, *Bacopa monnieri* has emerged as a promising natural alternative with the potential to improve brain function (Simpson et al., 2015). Studies suggest it acts as an antioxidant, reducing free radical damage in the brain and exhibiting neuroprotective effects (Singh B et al., 2021)

In the current investigation, we screened the 32 saponins of *B. monnieri* and successfully identified its 13 targets/proteins involved in cognitive impairment. Afterwards, network analysis suggested that these targets of BM have close association with Cognition disorders, Brain diseases, Neurodevelopmental disorders, Neurodegenerative disorder, Dementia and

Alzheimer's disease. By constructing a 'compound-target-disease' network, we establish a framework to understand the complex mechanisms through which saponins may interact with multiple targets and pathways relevant to cognitive decline. This approach may align with drug repurposing and identifying connections between saponins and existing drugs to accelerate treatment development for cognitive impairment.

The gene ontology analysis shows that the highly enriched molecular function was voltage gated sodium channel. Voltage-gated sodium channels (VGSCs) are crucial players in the realm of excitability in various cell types, particularly neurons. Voltage-gated sodium channels (VGSCs) act as microscopic gateways in cell membranes, regulating sodium ion flow. In humans, nine closely related genes encode VGSCs, including Nav1.1, Nav1.2, Nav1.3, and Nav1.6 proteins, pivotal for initiating and propagating action potentials in neural networks (Barbieri et al., 2023). Studies by Busche et al. linked Alzheimer's disease (AD) pathophysiology to seizures and neuronal hyperexcitability, potentially induced by elevated Aβ oligomers. Various pieces of evidence suggest that neuronal hyperactivity induced by amyloid-β1–42 (Aβ1–42) may contribute to cognitive impairments and memory dysfunction in AD (Busche et al., 2012; Ciccone et al., 2019). Yuan et al., (2022) found increased Nav1.6 expression in aged AD mice, leading to heightened BACE1 transcription, crucial for amyloid plaque formation. Therefore, targeting the molecular function of voltage-gated sodium channels may hold promise as a potential intervention strategy for Alzheimer's disease progression.

The analysis of pathway enrichment indicates that the 13 shared targets of Saponin are linked to pathways including the HIF-1 signaling pathway and the JAK-STAT signaling pathway. The HIF-1 (Hypoxia-Inducible Factor 1) pathway responds to low oxygen levels in the body. Its role in neurodegeneration remains unclear, but studies suggest that decreased oxygen supply

could both cause and result from neurodegeneration during aging. Aβ stimulates HIF-1α expression and activity, with minimal Aβ levels protecting neurons by activating HIF-1α pathways. However, Alzheimer's disease (AD) brains show decreased HIF-1 levels, potentially harming crucial HIF target genes (Mateo et al., 2006; Josepha et al., 2001). The JAK/STAT pathway is essential in the central nervous system, influencing neurogenesis, gliogenesis, synaptic plasticity, and microglia activation. Dysregulation of this pathway, particularly through phosphorylation, is associated with various neurodegenerative diseases, including AD (Ding et al., 2022). *B. monnieri* saponins' potential interaction with these pathways suggests a significant impact on the nervous system. This implies their substantial role in supporting neuronal growth, survival, and overall functionality.

All phytochemicals underwent ADMET screening to assess drug-likeness properties, demonstrating adherence to primary criteria, indicating potential as natural inhibitors. Essential for neuroprotective drug development is the ability to cross the blood-brain barrier (BBB) (Stępnik K. 2021), with all saponins showing acceptable BBB values, suggesting candidacy as neuroprotective agents. Additionally, favorable bioavailability scores indicate their promising role as inhibitors. Toxicity assessments reveal minimal associated toxicity with these phytochemicals.

The molecular docking studies revealed that saponins shows a significant binding affinity with SCN2A, SCN3A, DNMT1, MTOR. The mechanistic target of rapamycin (mTOR) plays a significant role in fundamental cellular functions such as metabolism and differentiation. Activating mTOR in microglia could positively impact Alzheimer’s disease-related pathologies linked to β-Amyloid (Aβ). This activation enhances Trem2 expression, crucial for Aβ plaque removal, implying potential benefits for Alzheimer’s patients with Aβ-related pathology (Shi et al., 2022). DNA methylation and hydroxymethylation are vital processes in

the human brain, crucial for normal development and function, including neural stem cell activities, synaptic plasticity, and learning/memory. DNMT1, a key enzyme in these processes, influences brain development, ageing, and neurodegeneration-related processes. Research shows age-dependent declines in DNMT1 levels (Yan et al., 2023). SCN2A and SCN3A encodes sodium channel protein that is important for the development and function of neurons.

Molecular dynamic simulation indicated that the complex SCN2A-Bacoside A1 and SCN2A-Bacoside A2 remains stable throughout the 100ns simulation process, this stability is supported by various interactions such as hydrogen, hydrophobic bonds and salt bridges suggesting that the Bacoside A1 and Bacoside A2 two sponins present in BM may downregulate Voltage-gated sodium channels molecular function and can probably intervene in the progression of Alzhimer's disease and cognitive impairmennt. Other complexes formed between DNMT1 and saponins such as Bacoside A2, Bacopaside N1 and Bacopasaponin E remains significantly stable during the simulation period indicating their potential as potent drugs in cognitive impairment.

The diverse saponins in *B. monnieri* targets various brain disorder aspects, playing a pivotal role in neurodegenerative pathways and reducing cognitive impairment impact. ADMET, docking, and simulation results highlight their therapeutic potential for cognitive disorders. Further animal model studies could strengthen these findings, advancing drug development. Another potential avenue for future research may involve modifying saponin molecules to enhance efficacy and create more potent drugs. Exploring saponin derivatives with improved drug-like characteristics could target additional cognitive disease pathways.

Tables

Table 1: Library of Saponin (Ligand) prepared from literatures

Bacogenin A	Bacopaside II	Bacopaside VII
Bacomosaponin A	Bacopaside III	Bacopaside VIII
Bacomosaponin B	Bacopaside IV	Bacopaside X
Bacopasaponin C	Bacopaside IV	Bacopaside XI
Bacopasaponin D	Bacopaside IV	Bacopaside XII
Bacopasaponin E	Bacopaside IX	Bacoside A
Bacopasaponin F	Bacopaside N1	Bacoside A1
Bacopasaponin G	Bacopaside N2	Bacoside A2
Bacopaside A3	Bacopaside V	Bacoside A3
Bacopaside I	Bacopaside VI	Bacoside B
Bacosine	Bacosterol	

Table 2: Common Proteins obtained after comparing the lists of Predicted targets of Saponins and Proteins known to play role in CI

Target/Protein Name	**Protein Full form**
TLR4	Toll-like receptor 4
SCN2A	Sodium voltage-gated channel alpha subunit 2
THRB	Prothrombin
SCN3A	Sodium voltage-gated channel alpha subunit 3
CCR1	C-C chemokine receptor type 1
KIF11	Kinesin-like protein KIF11
DNMT1	DNA (cytosine-5)-methyltransferase 1
MTOR	Mammalian target of rapamycin
EP300	E1A binding protein p300 (p300)
HDAC4	Histone deacetylase 4
GBA1	Lysosomal acid glucosylceramidase
THIK	3-ketoacyl-CoA thiolase, peroxisomal
BCR	Breakpoint cluster region protein

Table 3: ADMET Analysis of Saponins present in *Bacopa monnieri.*

Phytochemical	**Caco-2 [log (cm/s)]**	**HIA [%]**	**LogP [log-ratio]**	**BBB [%]**	**Ames [%]**
Bacomosaponin A	-5.67	49.13	1.76	15.62	46.29
Bacomosaponin B	-5.71	49.48	1.72	15.5	45.46
Bacopasaponin C	-5.7	52.69	1.68	10.23	44.81
Bacopasaponin D	-5.64	58.06	1.82	16.57	42.67
Bacopasaponin E	-5.81	49.9	1.71	10.34	45.36
Bacopasaponin F	-5.88	48.74	1.67	11.7	47.03
Bacopasaponin G	-5.63	60.84	1.79	15.75	43.3
Bacopaside A3	-5.21	61.74	1.6	26.18	42.17
Bacopaside I	-5.75	54.1	1.72	15.21	47.36
Bacopaside II	-5.72	49.58	1.79	11.27	45.87
Bacopaside III	-5.6	55.16	1.69	13.56	47.16
Bacopaside IV	-5.59	60.84	1.76	14.2	43.13
Bacopaside IX	-5.62	50.05	1.72	13.76	43.06
Bacopaside N1	-5.62	57.6	1.82	12.62	41.72
Bacopaside N2	-5.62	56.43	1.8	14.25	42.82
Bacopaside V	-5.61	59.64	1.76	16.43	43.23
Bacopaside VI	-5.6	55.16	1.69	13.56	47.16
Bacopaside VII	-5.72	50.11	1.69	8.51	45.25
Bacopaside VIII	-5.88	48.74	1.67	11.7	47.03
Bacopaside X	-5.72	50.11	1.69	8.51	45.25
Bacopaside XI	-5.66	54.24	1.75	15.8	48.07
Bacopaside XII	-5.79	49.76	1.73	14.42	45.27
Bacoside A1	-5.62	60.84	1.79	15.75	43.3
Bacoside A2	-5.74	55.87	1.81	15.89	43.03
Bacoside A3	-5.72	49.73	1.75	10.06	44.18
Bacoside B	-5.53	59.15	1.86	15.11	40.67
Bacomosaponin A	-5.67	49.13	1.76	15.62	46.29

Table 4: Top 10 docking results from docking of ligands with common proteins.

Target/Protein	Ligand	Affinity (kcal/mol)	LLE	SILE
SCN2A	Bacoside A1	-11.33	5.523	3.463
DNMT1	Bacoside A2	-10.5	7.234	3.03
SCN2A	Bacopasaponin C	-10.46	7.204	3.018
SCN2A	Bacopasaponin B	-10.42	4.999	3.185
SCN3A	Bacopaside IV_1	-10.41	5.488	3.146
SCN2A	Bacoside A2	-10.34	7.116	2.983
SCN3A	Bacoside A2	-10.33	7.109	2.981
MTOR	Bacoside A2	-10.28	7.072	2.966
MTOR	Bacopaside IX	-10.03	7.075	2.758
SCN2A	Bacopasaponin G	-9.948	4.51	3.04

Table 5: Molecular docking of saponins from *B. monnieri* against the selected proteins

Target/Protein	Ligand	Binding Affinity (kcal/mol)	Interacting residue
SCN2A	Bacoside A1	-11.33	Lys1422, Gln383, Tyr1771, Gln383
DNMT1	Bacoside A2	-10.34	Glu703, Arg1311, Gln1300, Asn1270, Gln1227, Ser1230
SCN2A	Bacoside A2	-10.34	Lys1422, Ser1713, Gln383
DNMT1	Bacopaside N1	-9.53	Gly500, Asn543, Arg544, Thr546, Arg552
DNMT1	Bacopasaponin E	-9.07	Arg1310, Cys1226, Arg1311, Gln1300, Thr1309, Pro1224, Glu1266, Thr1309, Ser1524

Figures Legends

Figure 1: Venny diagram showing common proteins (13) associated with both saponins (present in BM*)* target proteins and the proteins involved in cognitive impairment

Figure 2: Dot plot and Bar plot of top 10 Biological Process, Cellular Components, Molecular Functions and Pathway analysis with their enrichment scores.

Figure 3: Cytoscape Network showing Brahmi, its Cognitive Impairment Targets (shown in purple) and various diseases associated with those targets (shown in green)

Figure 4: Molecular Docking region and various interactions that were formed between the saponins and the selected proteins.

Figure 5: Root mean deviation and protein-ligand interaction showing various bonds of protein with the top-hit saponins for a Molecular Simulation run for 100ns.

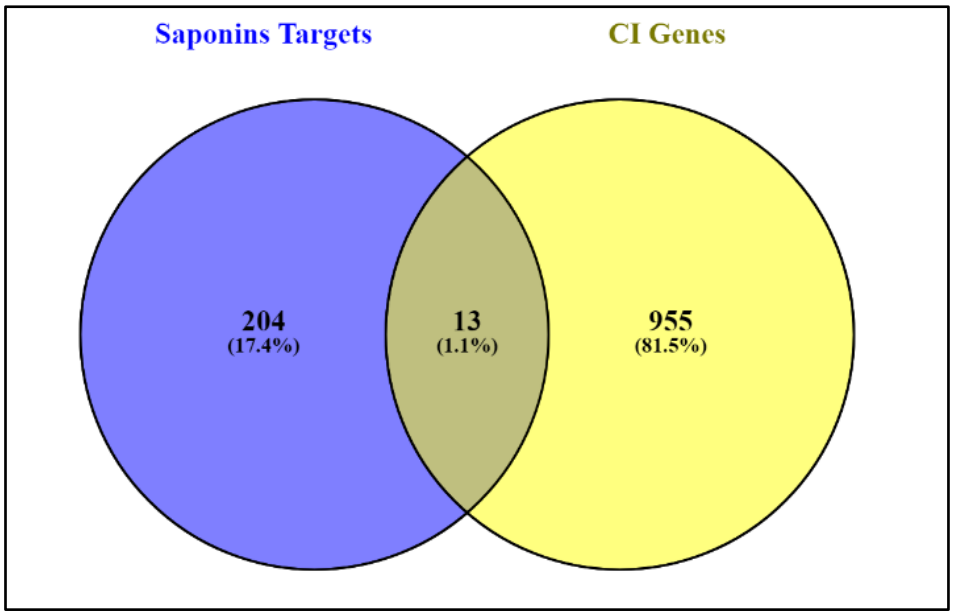

Figure 1: Venny diagram showing common proteins (13) associated with both saponins (present in BM*)* target proteins and the proteins involved in cognitive impairment

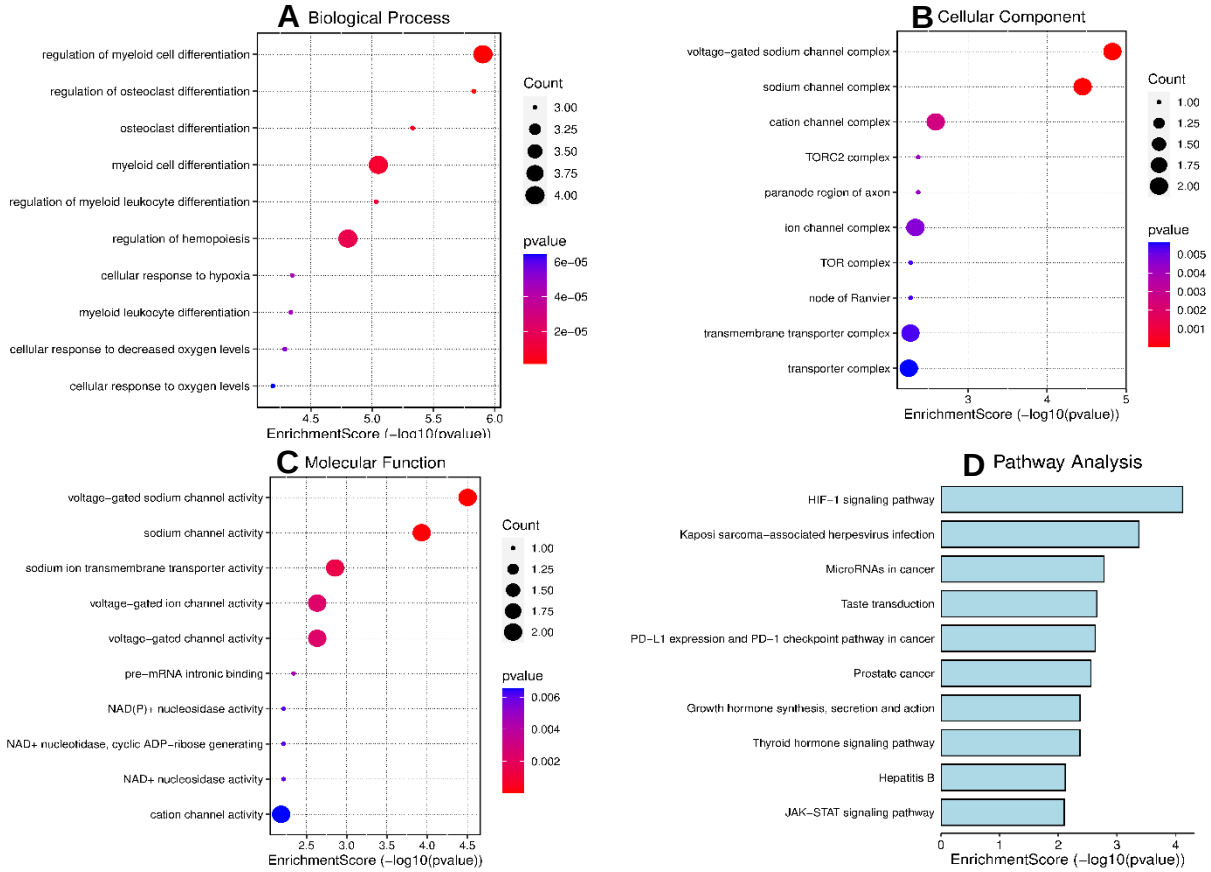

Figure 2: Dot plot and Bar plot of top 10 **A**. Biological Process, **B**. Cellular Components, **C**. Molecular Functions and **D**. Pathway analysis with their enrichment scores.

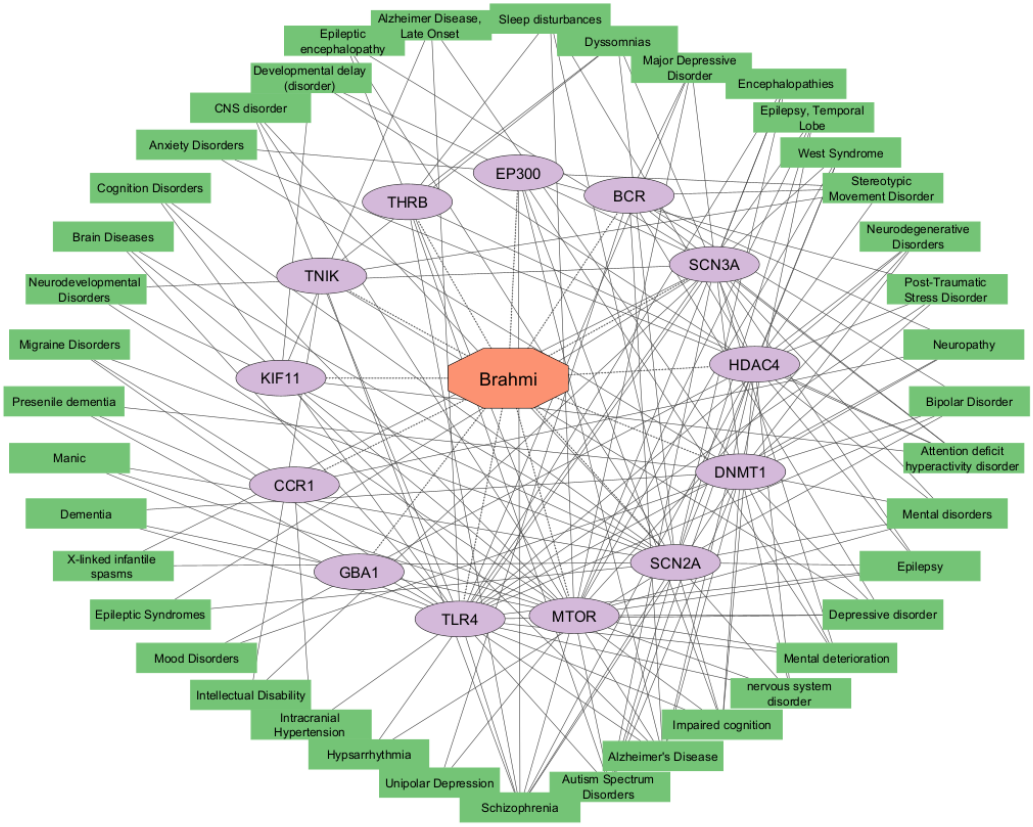

Figure 3: Cytoscape Network showing Brahmi, its Cognitive Impairment Targets (shown in purple) and various diseases associated with those targets (shown in green)

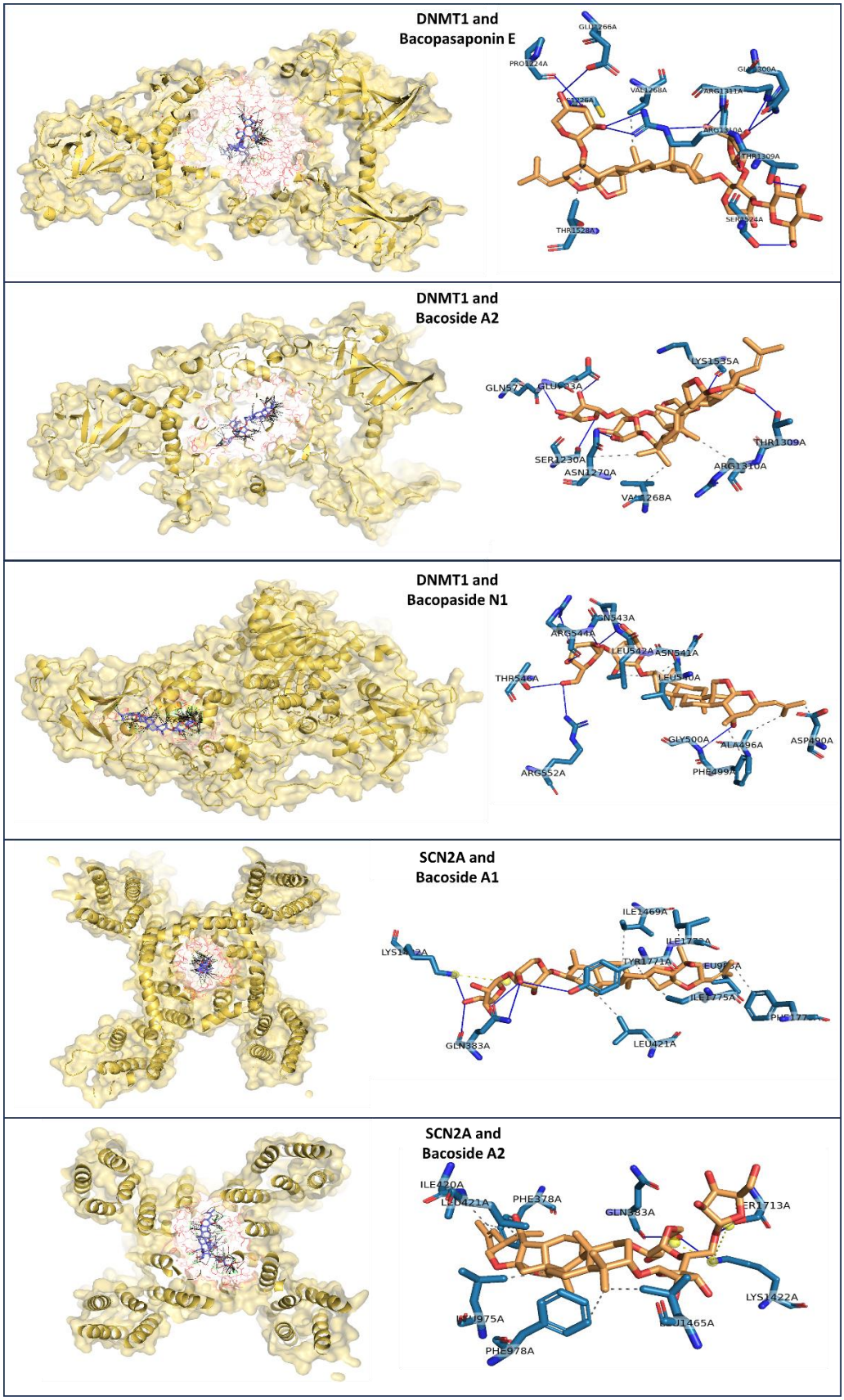

Figure 4: Molecular Docking region and various interactions that were formed between the saponins and the selected proteins.

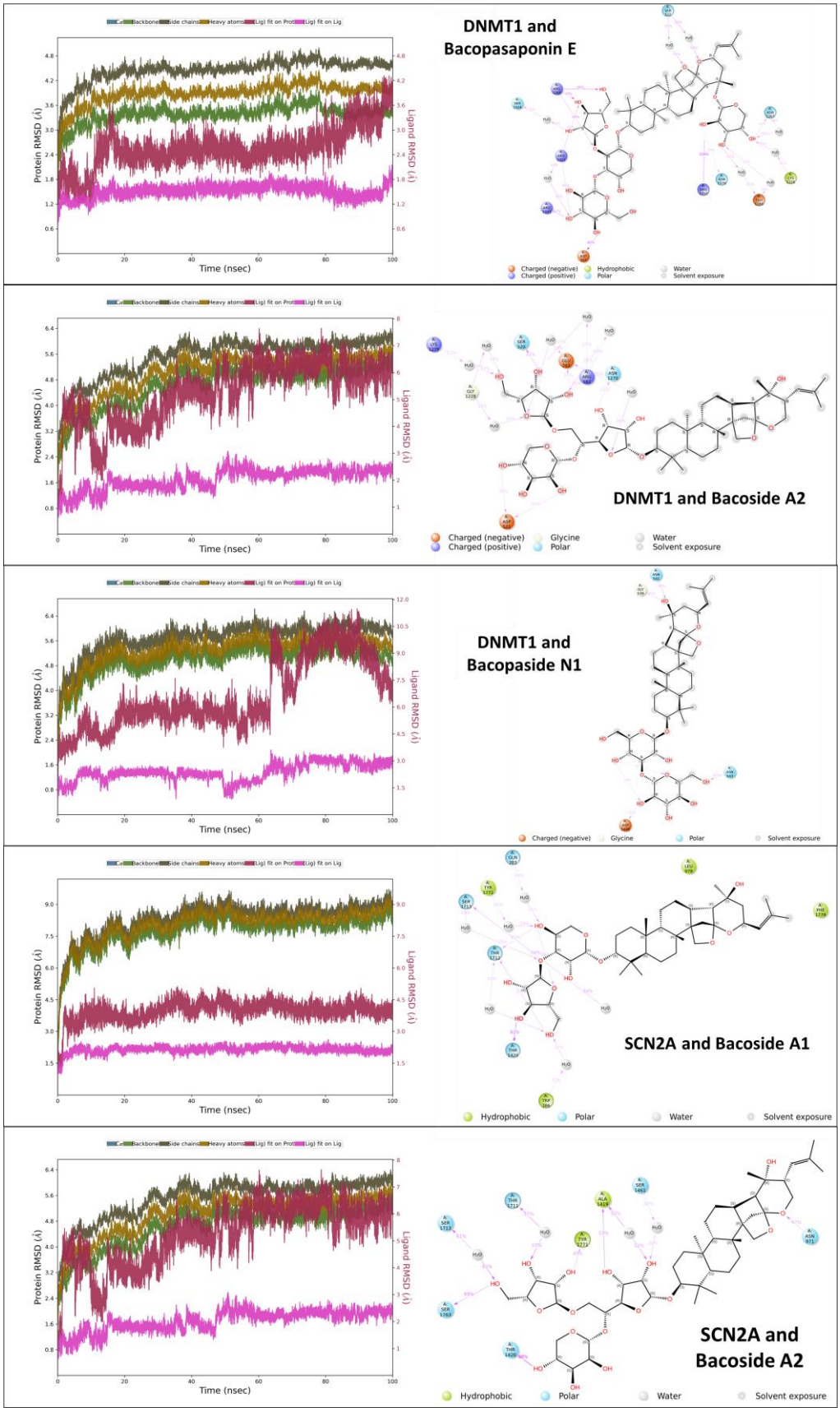

Figure 5: Root mean deviation and protein-ligand interaction showing various bonds of protein with the top-hit saponins for a Molecular Simulation run for 100ns.

References

Banerjee, S., Anand, U., Ghosh, S., Ray, D., Ray, P., Nandy, S., Deshmukh, G. D., Tripathi, V. and Dey, A. (2021). Bacosides from Bacopa monnieri extract: An overview of the effects on neurological disorders. *Phytotherapy research* : PTR, 35(10), 5668–5679.

Barbieri, R., Nizzari, M., Zanardi, I., Pusch, M. and Gavazzo, P. (2023). Voltage-gated sodium channel dysfunctions in neurological disorders. *Life*, 13(5), 1191.

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009 Apr 15;25(8):1091–3.

Bowers, K. J., Chow, E., Xu, H., Dror, R. O., Eastwood, M. P., Gregersen, B. A., ... and Shaw, D. E. (2006, November). Scalable algorithms for molecular dynamics simulations on commodity clusters. *In Proceedings of the 2006 ACM/IEEE Conference on Supercomputing* (pp. 84-es).

Busche, M. A., Chen, X., Henning, H. A., Reichwald, J., Staufenbiel, M., Sakmann, B. and Konnerth, A. (2012). Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences*, 109(22), 8740-8745.

Ciccone, R., Franco, C., Piccialli, I., Boscia, F., Casamassa, A., de Rosa, V., Cepparulo, P., Cataldi, M., Annunziato, L. and Pannaccione, A. (2019). Amyloid β-induced upregulation of Nav1. 6 underlies neuronal hyperactivity in Tg2576 Alzheimer's disease mouse model. *Scientific reports*, 9(1), 13592.

Delfan, M., Kordestani-Moghaddam, P., Gholami, M., Kazemi, K. and Mohammadi, R. (2024). Evaluating the effects of Bacopa monnieri on cognitive performance and sleep

quality of patients with mild cognitive impairment: A triple-blinded, randomized, placebo-controlled trial. *Explore (New York, N.Y.)*, S1550-8307(24)00053-3.

Ding, M. R., Qu, Y. J., Hu, B. and An, H. M. (2022). Signal pathways in the treatment of Alzheimer's disease with traditional Chinese medicine. *Biomedicine and Pharmacotherapy*, 152, 113208.

Du, L., Geng, C., Zeng, Q., Huang, T., Tang, J., Chu, Y. and Zhao, K. (2023). Dockey: a modern integrated tool for large-scale molecular docking and virtual screening. *Briefings in Bioinformatics*, 24(2), bbad047.

Dubey, T. and Chinnathambi, S. (2019). Brahmi (Bacopa monnieri): An ayurvedic herb against the Alzheimer's disease. *Archives of biochemistry and biophysics*, 676, 108153.

Fatima, U., Roy, S., Ahmad, S., Al-Keridis, L. A., Alshammari, N., Adnan, M., Islam, A. and Hassan, M. I. (2022). Investigating neuroprotective roles of Bacopa monnieri extracts: Mechanistic insights and therapeutic implications. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 153, 113469.

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E. and Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization and analysis platform. *Journal of cheminformatics*, 4, 1-17.

Hopkins, A. L., Keserü, G. M., Leeson, P. D., Rees, D. C. and Reynolds, C. H. (2014). The role of ligand efficiency metrics in drug discovery. *Nature reviews Drug discovery*, 13(2), 105-121.

Jaiswal, Y. S. and Williams, L. L. (2016). A glimpse of Ayurveda - The forgotten history and principles of Indian traditional medicine. *Journal of traditional and complementary medicine*, 7(1), 50–53.

Jeyasri, R., Muthuramalingam, P., Suba, V., Ramesh, M. and Chen, J. T. (2020). Bacopa monnieri and their bioactive compounds inferred multi-target treatment strategy for

neurological diseases: A cheminformatics and system pharmacology approach. *Biomolecules*, 10(4), 536.

Josepha, J., Shukitt-Hale, B., Denisova, N. A., Martin, A., Perry, G. and Smith, M. A. (2001). Copernicus revisited: amyloid beta in Alzheimer's disease. *Neurobiology of aging*, 22(1), 131-146.

Joshi, C., Chaudhari, A., Joshi, C., Joshi, M. and Bagatharia, S. (2022). Repurposing of the herbal formulations: molecular docking and molecular dynamics simulation studies

Kenny, P. W. (2019). The nature of ligand efficiency. *Journal of Cheminformatics*, 11, 1-18.

Malík, M. and Tlustoš, P. (2023). Nootropic Herbs, Shrubs, and Trees as Potential Cognitive Enhancers. *Plants (Basel, Switzerland)*, 12(6), 1364.

Malík, M., & Tlustoš, P. (2022). Nootropics as cognitive enhancers: types, dosage and side effects of smart drugs. *Nutrients*, *14*(16), 3367.

Mateo, I., Llorca, J., Infante, J., Rodríguez-Rodríguez, E., Sánchez-Quintana, C., Sánchez-Juan, P., Berciano, J and Combarros, O. (2006). Case-control study of vascular endothelial growth factor (VEGF) genetic variability in Alzheimer's disease. *Neuroscience letters*, 401(1-2), 171-173.

Pajouhesh, H. and Lenz, G. R. (2005). Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*, 2, 541-553.

Pérez, M. A. C., Sanz, M. B., Torres, L. R., Ávalos, R. G., González, M. P. and Díaz, H. G. (2004). A topological sub-structural approach for predicting human intestinal absorption of drugs. *European journal of medicinal chemistry*, 39(11), 905-916.

Shankar D. (2023). Reimagining India's national health system (NHS). *Journal of Ayurveda and integrative medicine*, 14(1), 100663.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. Nov;13(11):2498–504.

Shi, Q., Chang, C., Saliba, A. and Bhat, M. A. (2022). Microglial mTOR activation upregulates Trem2 and enhances β-amyloid plaque clearance in the 5XFAD Alzheimer's disease model. *Journal of Neuroscience*, 42(27), 5294-5313.

Simpson, T., Pase, M., & Stough, C. (2015). Bacopa monnieri as an antioxidant therapy to reduce oxidative stress in the aging brain. *Evidence-based complementary and alternative medicine*, *2015*.

Singh, A., Dauzhenka, T., Kundrotas, P. J., Sternberg, M. J. E. and Vakser, I. A. (2020). Application of docking methodologies to modeled proteins. *Proteins*, 88(9),

Singh, B., Pandey, S., Rumman, M., Kumar, S., Kushwaha, P. P., Verma, R. and Mahdi, A. A. (2021). Neuroprotective and neurorescue mode of action of Bacopa monnieri (L.) Wettst in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease: an in silico and in vivo study. *Frontiers in Pharmacology*, 12, 616413.

Stępnik K. (2021). Biomimetic Chromatographic Studies Combined with the Computational Approach to Investigate the Ability of Triterpenoid Saponins of Plant Origin to Cross the Blood-Brain Barrier. *International journal of molecular sciences*, 22(7), 3573.

Yan, L., Geng, Q., Cao, Z., Liu, B., Li, L., Lu, P. and Lu, C. (2023). Insights into DNMT1 and programmed cell death in diseases. *Biomedicine and Pharmacotherapy*, 168, 115753.

Yuan, D. J., Yang, G., Wu, W., Li, Q. F., Xu, D. E., Ntim, M., Jiang, C. Y., Liu, J. C., Zhang, Y., Wang, Y. Z., Zhu, D. D., Kundu, S., Li, A. P., Xiao, Z. C., Ma, Q. H. and Li,

S. (2022). Reducing Nav1. 6 expression attenuates the pathogenesis of Alzheimer's disease by suppressing BACE1 transcription. *Aging Cell*, 21(5), e13593.

Zhang, W., Zeng, Y., Jiao, M., Ye, C., Li, Y., Liu, C. and Wang, J. (2023). Integration of high-throughput omics technologies in medicinal plant research: The new era of natural drug discovery. *Frontiers in plant science*, 14, 1073848.