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Molecular Modeling Tools Used for the Prediction of Amyloid-β Fibrils Disaggregating Molecules from Plant Sources

Uthirapathi Logeswari Rakesh [1], Golla Anil Kumar [1], Theivendren Panneerselvam [2], Parasuraman Pavadai [3], Suganthan Veerachamy [4], Ponnusamy Palanisamy [4], Sunil Kumar Bandral [3], Alagarsamy Santhana Krishna Kumar [5,*] and Selvaraj Kunjiappan [1,*]

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Abstract: The defining characteristic of Alzheimer's disease (AD) is the aberrant deposition of pathogenic proteins, particularly a massive build-up of amyloid-β peptides, specifically in the brain. Amyloid-β aggregation is neurotoxic and ultimately results in dysfunction of the nervous system. The present investigation aims to predict potential amyloid-β fibrils disaggregating molecules from plant sources through molecular modeling techniques, this appears to be a very promising and appealing treatment philosophy. Here, 500 flavonoids from various plants were considered, initially undergoing ADME studies to screen molecules that could cross the blood-brain barrier. Later, potential Amyloid-β-disaggregating molecules were identified by molecular docking and dynamics (MD) simulation studies. Five molecules, prenylmethoxy flavonol (-7.3 kcal×mol^{-1}), isopentenyl flavonol (-7.3 kcal×mol^{-1}), 7,3'-Dihydroxyflavone (-7.2 kcal×mol^{-1}), 7-Hydroxy-5-methyl-4'-methoxyflavone (-7.2 kcal×mol^{-1}), 8-hydroxy-7-methoxyflavone (-7 kcal×mol^{-1}) showed higher binding affinity score against Alzheimer's Aβ (1-42) fibrils, these are very close to the standard drug (Donepezil) (-7.90 kcal×mol^{-1}). Further, the MD simulation studies established the intermolecular interaction and stability of the five selected ligands-Amyloid-β oligomer protein complexes. These results suggest that the chosen five flavonoid molecules could be used as possible agents to disaggregate amyloid-β fibrils; however, more in vitro and in vivo investigations are required to verify the therapeutic effectiveness.

Keywords: Alzheimer's disease; amyloid-β fibrils; flavonoids; disaggregation; blood-brain barrier

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative condition connected with verbal, behavioral, and cognitive problems that eventually interfere with day-to-day functioning [1]. Sadly, there is no known treatment for AD, and each person's experience with the disease is unique [2]. The deposition of abnormal proteins within and around brain cells is thought to be the root cause of AD [3]. Amyloid-beta (Amyloid-β) is a naturally occurring protein that accumulates in the Alzheimer's brain in abnormal proportions to form plaques that damage cell function [4]. Amyloid-β plaques are the main factor behind neuritic plaques in AD, which gradually deteriorates cognitive abilities [5]. Amyloid-β monomers (37–42 residues long) are released when the enzyme Amyloid Precursor Protein (APP) breaks down. These monomers aggregate to create neurotoxic Amyloid-β fibrils [6]. The early formation of Amyloid-β plaques mostly depends on the monomeric Amyloid-β42 peptide [7]. The misfolded Amyloid-β42 peptides undergo self-assembly to generate oligomers, which then combine

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to form fibrils [8]. The Amyloid-β42 monomer is a crucial indicator of AD and has been extensively utilized in preventing and managing AD.

Two potential strategies exist to prevent the folding error and accumulation of the Amyloid-β42 peptide. The first is limiting the Amyloid-β monomer's conformational change using inhibitors targeting this process. The second approach is to destabilize/disaggregate the native state of the Amyloid-β42 monomer by refolding it from its misfolded configuration [9]. Researchers initiated destabilization/disaggregation as a therapeutic method due to the clinical failure of medicines using an anti-aggregation technique [10]. Amyloid-β fibril destabilization has a double effect: the distorted fibrils stop being neurotoxic in and of themselves and prevent the production of additional higher-order aggregates [11]. Natural chemicals have been the go-to option for breaking up preformed Amyloid-β fibrils because of their minimal toxicity to humans and biocompatibility [12]. Plant-based phytochemicals are the safest option among all-natural compound sources because of their greater potency, fair availability, extractability, and broad-spectrum applicability. Natural polyphenolic molecules, especially flavonoids, are gaining much attention because they inhibit the formation and aggregation of Amyloid-β fibrils [13].

Plants contain a class of secondary metabolites called flavonoids, which have attracted the pharmaceutical and healthcare industries' interest because of their numerous potential therapeutic applications. Flavonoids comprise a benzopyrone ring, including additional phenolic or polyphenolic groups at various locations [14]. These are produced spontaneously via the phenylpropanoid pathway, and their bioactivity relies on their bioavailability and mode of absorption. Flavanoid compounds are present in most plants, fruits, herbs, stems, cereals, nuts, vegetables, flowers, and seeds [15]. To date, over 10,000 distinct flavonoid molecules have been identified and extracted. Most flavonoids are widely acknowledged to be helpful therapeutic agents, including neuroprotection [16]. The development of AD is believed to depend on an overproduction of reactive oxygen species (ROS), which leads to neurofibrillary tangles as tau aggregates and the aggregation of amyloid-β plaques [17]. This can also lead to higher metabolic demand and mitochondrial dysfunction [18]. Some studies have suggested flavonoids may offer protective effects for brain cells [19]. Some early-stage animal studies have shown that flavonoids can block amyloid-β plaque buildup in the brain, a trademark of Alzheimer's [20]. These naturally occurring flavonoids were suggested as possible therapeutic candidates, but their molecular mechanism remains unclear, thus, it would be ideal to increase in silico research.

The technique of using in silico molecular modeling research to discover new drug candidates from plant-derived flavonoids for AD treatment is outstanding and continually changing. There is an increasing agreement that plant-derived flavonoids with aromatic rings are suitable for treating AD. Flavonoids have been found to possess antioxidant and neuroprotective properties, which have been shown to slow down the onset of AD [21]. Molecular dynamics simulation modeling provides atomistic insights into the mechanism of disaggregating Amyloid-β fibrils, which can aid in discovering drugs for treating AD [22]. In this work, five hundred diverse flavonoid compounds derived from plants were randomly chosen from drug libraries. The objective was to anticipate probable molecules that can disaggregate amyloid-β fibrils. The molecules were picked randomly, and their ability to penetrate the blood-brain barrier was determined using in silico ADME analysis. Additionally, these flavonoids were docked with Amyloid-β oligomer to identify potential binders of Amyloid-β oligomer. The screened complexes were further subjected to molecular dynamics (MD) modeling to identify the most promising molecules for the disaggregation of Amyloid-β fibrils.

2. Experimental Section

2.1. Amyloid-β Protein Preparation

The 3-dimensional structure of Alzheimer's amyloid-beta (1-42) fibrils (PDB entry id: 2BEG, Homo sapiens) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/structure/2BEG). Before molecular docking studies, the retrieved amyloid-β (1-42) fibrils were pre-processed through Swiss-PDB Viewer v4.1.0, which included the addition of polar hydrogens and Kollmann charges and removing water molecules. The file was then designated

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as target.pdb and kept for further investigations. A grid box was created based on the outcomes of the ligand binding site prediction tool of PrankWeb (https://prankweb.cz/). The active sites' protein structure and amino acid locations were determined using BIOVIA Discovery Studio Visualiser version 4.0 software (Accelry's Software Inc., San Diego, CA).

2.2. Ligands Selection

Five hundred flavonoid molecules were chosen from different plant sources from IMPPAT (https://cb.imsc.res.in/imppat/) [23] and Dr. Duke's databases (https://phytochem.nal.usda.gov/) [24]. The molecular structures of selected flavonoid molecules and standard drug (Donepezil) were drawn using Chemsketch software and exported as Structure Data Format (SDF). They were then converted using Open Babel to the mol2 chemical representation. Atoms were allocated Gasteiger-type polar hydrogen charges, whereas non-polar hydrogen molecules were combined with carbons. The internal degrees of freedom and torsions were then adjusted to zero. The ligand molecules were subsequently transformed into the dockable PDBQT format using AutoDock Tools.

2.3. In Silico ADME Screening

The blood-brain barrier, or BBB, keeps larger substances and undesirable cells out of the brain while selectively allowing ions, nutrition, and small molecules (less than 400 Da) to enter [25]. Consequently, the SwissADME web tool (https://www.swissadme.ch) was used to anticipate possible BBB crossing compounds from the five hundred compounds that were previously selected. In this regard, SMILES (Simplified Molecular Input Line Entry System) was translated into their 3D structure using an online SMILES translator and structure file generator (https://cactus.nci.nih.gov/translate/).

2.4. Active site-Targeted Molecular Docking of Flavonoids

The previously stated Swiss-ADME study screened the flavonoid molecules selected for molecular docking studies. These investigations aim to identify compounds with a solid capacity to break down amyloid-β fibrils. Using the AutoDock Vina tool in PyRx 0.8 software, the chosen compounds and the conventional medication donepezil were docked against amyloid-β fibrils (PDB id: 2BEG), emphasizing the active amino acid residues. The molecules and the standard medication, donepezil, were imported using the Open Babel tool, and then the energy was minimized. Then, PyRx 0.8 was loaded with the optimised compounds. Conjugate gradient descent was the energy optimization algorithm, while the Universal Force Field (UFF) was the energy minimization parameter. The amyloid-β fibrils (2BEG) active region was used for the docking studies. It had a grid box size of 30.34 × 25.0 × 18.93 Å and was centered at coordinates (x, y, z) of (-5.73, 0.024, 0.247) Å. The remaining parameters were all kept at their original configurations. The Discovery Studio Visualiser version 16 was used to observe the intermolecular interactions.

2.5. Molecular Dynamics (MD) Simulation Studies

The stability of binding, confirmation, and intermolecular interactions between selected highly ranked bioactive molecules (ligands) and the target protein, amyloid-β fibrils (2BEG), were investigated using MD simulation studies. A 100-nanosecond time-dependent evolution of the complexes was estimated on a Linux system using the Desmond dynamic package 2017 in Schrodinger (academic version). Under physiological settings, the MD simulation (Schrodinger, LLC, Schrodinger Release: QikProp) highlights the likely outcomes of protein-ligand complexes (PLCs) at target binding sites. The system developers' panel: Initially, the panel lets us build a cubic (10 × 10 × 10) container that can house physiological parameters like pH and molecules of water. Na+ or Cl- ions can be added if the pH is low or needs to be raised to satisfy the particular demands of the research methodology. The orthorhombic point-charge (SPC) water model was used to solve the docked protein-ligand complexes straightforwardly. Counter ions were added to the solvated system to make it electrically neutral while keeping the physiological salt concentration at 0.15 M [26]. The OPLS AA (Optimal Potentials for Liquid Simulation - All Atom) force field was applied to the PLC

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system [27]. In about 100 picoseconds, the system builder panel will moderately reduce the prepared PLC. Two picoseconds were used as the relaxation length in the MD simulation. The Nose-Hoover chain thermostat, the Martyna-Tobias-Klein barostat, and the Reversible Reference System Propagator Algorithms (RESPA) integrator were utilised [28]. The MD simulation's last iteration was produced with the system in equilibrium. The NPT ensemble was used for the MD simulation, which keeps the temperature, pressure, and particle count constant [29]. Using the default relaxation parameters, the simulation ran for 100 nanoseconds at a temperature of 310.15 Kelvin and a pressure of 1.0 bar. After the experiment, the results were assessed using a simulated interaction diagram.

2.6. Density Functionality Theory (DFT)

One well-known and valid "ab initio" technique for examining the structure of quantum many-body systems, such as atoms, molecules, and solids, is DFT [30]. The DFT analysis of the highest-scoring compounds was carried out using the Gaussian 03W package and the Gauss View molecular visualization program. They used a DFT/Becke-3–Lee–Yang–Parr (B3LYP)/6-311G (d, p) technique to ascertain the chosen bioactive chemicals' ideal molecular structure and vibrational frequencies. Furthermore, the optimal configurations and border molecular orbital energies of specific bioactive compounds were calculated. The energies are comprised of the energy difference (Eg) between the highest occupied orbital (EHOMO) and the lowest unoccupied orbital (ELUMO) of a macromolecule. The molecular orbital energy diagrams of the chosen bioactive compounds were examined using the Gauss View molecular visualization software.

3. Result and Discussion

3.1. Binding Site Identification

The binding site can be found by evaluating the physicochemical and shape characteristics of the protein region. To create novel drugs based on the target protein's structure, molecular docking requires discovering new chemical entities, which is vital. In this case, the Alzheimer's amyloid-β (1-42) fibrils' three-dimensional structure contains ten possible binding sites that the PrankWeb tool found (PDB entry id: 2BEG). The binding of amino acid residues to amyloid-β fibrils is shown in Figure 1. The 10 binding pockets were identified by a spectrum of colours, including red, light-yellow, dark-yellow, light green, dark green, light blue, tan, grey, pink, light orange, and dark bluish-green. The same binding pockets of the amyloid-β fibrils were utilized in the molecular docking process to screen the selected active compounds. The ligand posture grading accuracy was enhanced by molecular docking grid generation. We developed a receptor grid tailored to the chosen amyloid-β fibrils to improve our ligand posture score accuracy. The binding site residues discovered in earlier studies created this grid. With the box dimensions of X = 67.1909, Y = 91.6671, and Z = 88.4240 in angstrom (Å), a receptor grid was made to achieve this.

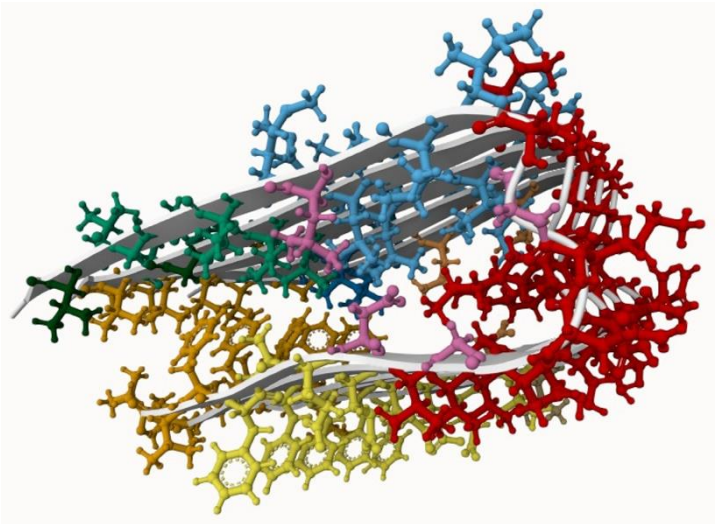

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Figure 1. The PrankWeb tool is used to predict the binding pockets and correspondence binding sites of the Alzheimer's amyloid-β (1-42) fibrils. The 10 binding pockets were distinguished by various colours: red, light-yellow, dark-yellow, light green, dark green, light blue, tan, grey, pink, light orange, and dark bluish-green.

3.2. Active Molecules and Their Structures

The conventional medication, donepezil, and the three-dimensional structures of 500 active compounds from different plants were obtained and optimized from the IMPPAT and Dr. Dukes databases. The results of in silico ADME and molecular docking analyses utilizing the optimized structures against the Alzheimer's amyloid-β (1-42) fibrils are shown in Supplementary Table 1.

3.3. In Silico ADME Screening

The blood-brain barrier (BBB) impedes the entry of most medications into the brain. The existence of the BBB hinders the discovery of drugs for Alzheimer's disease (AD). The BBB prevents over 98% of small-molecule medications and almost 100% of large-molecule pharmaceuticals from entering the brain. While most drug candidates for Alzheimer's disease (AD) are unable to pass through the blood-brain barrier (BBB), the current focus of AD drug development is heavily skewed towards discovering drugs that target the central nervous system (CNS), with less than 1% of the effort dedicated to developing drugs that can effectively reach the CNS [25]. Through ADME analysis, this study screened 125 blood-brain barrier crossing ability of flavonoids from 500 molecules. Supplementary Table 2 displays the chosen molecules for subsequent molecular docking.

3.4. Molecular Docking

Molecular docking analyses were conducted to find possible candidates for Alzheimer's amyloid-β (1-42) fibril disaggregation. Using AutoDock Vina, the binding affinity of 125 active compounds and the conventional medication donepezil to the disaggregation of amyloid-β fibrils was assessed to establish their potential for protein interaction with the target. The study revealed that five active compounds had the lowest binding energy (-7.3 kcal×mol^{-1}) against Alzheimer's amyloid-β (1-42) fibrils, the target protein. Furthermore, it was observed that the active compounds displayed a spectrum of binding energies, which varied from -7.7 to -5 kcal×mol-1, as illustrated in supplementary Table 2. Five molecules, namely prenylmethoxy flavonol (-7.3 kcal×mol^{-1}), isopentenyl flavonol (-7.3 kcal×mol^{-1}), 7,3'-Dihydroxyflavone (-7.2 kcal×mol^{-1}), 7-Hydroxy-5-methyl-4'-methoxyflavone (-7.2 kcal×mol^{-1}), and 8-hydroxy-7-methoxyflavone (-7 kcal×mol^{-1}), were selected for further investigation due to their potentially binding with the amino acid residues in the active site of amyloid-β (1-42) fibrils. The outcomes are also consistent with the publication [31], in which it was discovered that docking a flavonoid derivative (63), along with other flavones, destabilized the Aβ oligomer [31]. Compared to previous results, the least binding energy value of ΔG indicates that it would make an excellent binder to Aβ fibril [32]. The benchmark binding energy for the medication donepezil was -7.9 kcal×mol^{-1}. Because donepezil effectively breaks down the build-up of amyloid-β (1-42) fibril protein in the human brain, it was selected as the standard medication.

3.5. Interpretation of Ligands-Amyloid-β Fibrils Interactions

The protein-ligand interaction profiler web tool (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) was used to visualize the developed residue interactions between the chosen ligands with amyloid-β fibrils. One of the top-scored molecules, prenylmethoxy flavonol, binds with the target amyloid-β fibrils with the binding energy -7.3 kcal×mol^{-1}. The active molecule, prenylmethoxy flavonol, binds with amyloid-β fibrils protein to create four hydrogen bonds (27B ASN (2.95Å), 27C ASN (2.07Å), 27B ASN (2.79Å), and 29C GLY (2.04Å)), 2D and 3D interactions presented in Figure 2(a) and 2(b). The molecule, isopentenyl flavonol, binds with the target amyloid-β fibrils with the binding energy of -7.3 kcal×mol^{-1}. The isopentenyl flavonol binds with amyloid-β fibrils protein to create two hydrophobic interactions (30A ALA (3.84 Å) and 30B ALA (3.76Å)) and one hydrogen bonding (27C ASN (1.99 Å)), 2D and 3D interactions presented in Figure 2(c) and 2(d). The 7,3'-

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Dihydroxyflavone molecule is also docked with amyloid-β fibrils protein, with a binding energy of -7.2 kcal×mol^{-1}. 7,3'-Dihydroxyflavone formed one hydrophobic interaction (30C ALA (3.88Å)) and four hydrogen bonding (27B ASN (2.82 Å), 27C ASN (1.77 Å), 27D ASN (2.32 Å), and 30D ALA (2.61 Å)) with the target protein amyloid-β fibrils, 2D and 3D interactions presented in Figure 2(e) and 2(f.) The molecule 7-Hydroxy-5-methyl-4'-methoxyflavone binds with the target amyloid-β fibrils with the binding energy of -7.2 -7.2 kcal×mol^{-1}. The 7-Hydroxy-5-methyl-4'-methoxyflavone bind with amyloid-β fibrils protein to create two hydrophobic interactions (30C ALA (3.96 Å) and 30D ALA (3.72 Å)) and three hydrogen bonding (27B ASN (3.15 Å), 27D ASN (2.14 Å), and 30D ALA (2.58 Å)), 2D and 3D interactions presented in Figure 2(g) and 2(h). The 8-hydroxy-7-methoxyflavone molecule binds with the target amyloid-β fibrils with the binding energy of -7 -7.2 kcal×mol^{-1}. The 8-hydroxy-7-methoxyflavone bind with amyloid-β fibrils protein to create two hydrophobic interactions (30B ALA (3.90 Å) and 30C ALA (3.65 Å)) and five hydrogen bonding (27B ASN (3.03 Å), 27B ASN (2.30 Å), 27C ASN (2.24 Å), 30C ALA (3.11 Å) and 30D ALA (2.13 Å)), 2D and 3D interactions presented in Figure 2(i) and 2(j). The standard drug, Donepezil, binds with the target amyloid-β fibrils with a binding energy of -7.9 -7.2 kcal×mol^{-1}. The Donepezil binding with amyloid-β fibrils protein created two hydrophobic interactions (30B ALA (3.75 Å) and 30D ALA (3.60 Å)) and two hydrogen bonding (27D ASN (3.11 Å), and 30B ALA (2.61 Å)), 2D and 3D interactions presented in Figure 2(k) and 2(l).

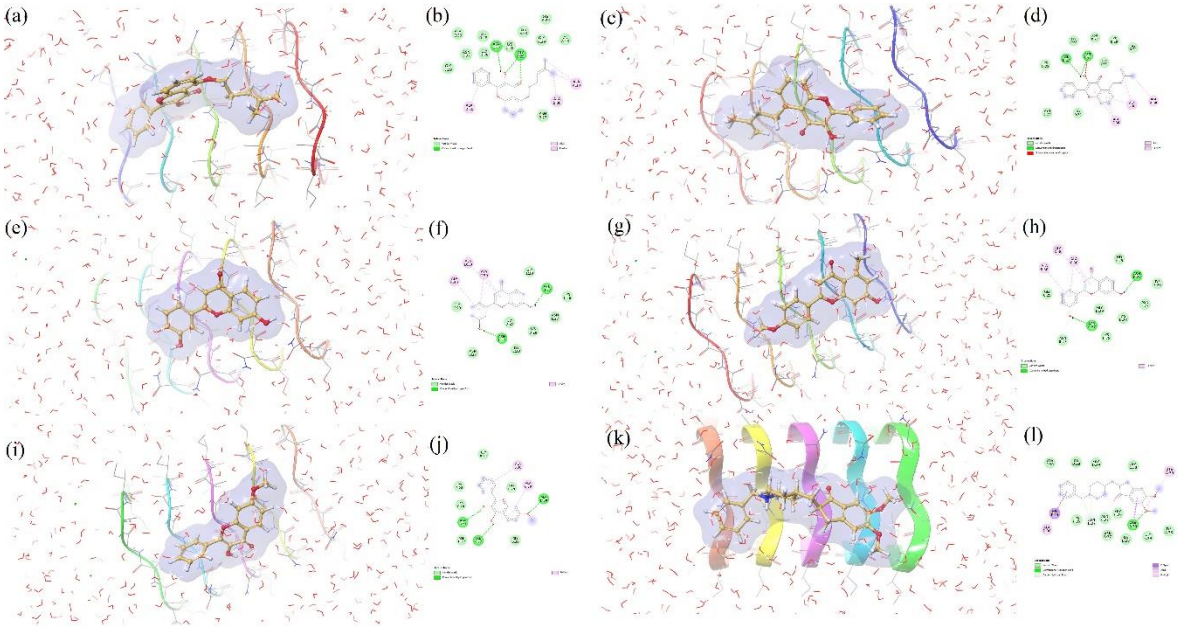

Figure 2. Interaction between the molecule Prenylmethoxy flavonol and Amyloid-β fibrils. Left side representing 3D (a) and the right side representing 2D complex protein–ligand interaction (b); the interaction between the molecule Isopentenyl flavonol and Amyloid-β fibrils. Left side representing 3D (c) and the right side representing 2D complex protein–ligand interaction (d); and the interaction between the molecule 7,3'-Dihydroxyflavone and Amyloid-β fibrils. Left side representing 3D (e) and the right side representing 2D complex protein–ligand interaction (f); and the interaction between the molecule 7-Hydroxy-5-methyl-4'-methoxyflavone and Amyloid-β fibrils. Left side representing 3D (g) and the right side representing 2D complex protein–ligand interaction (h); and the interaction between the molecule 8-hydroxy-7-methoxyflavone and Amyloid-β fibrils. Left side representing 3D (i) and the right side representing 2D complex protein–ligand interaction (j); and the interaction between the molecule Donepezil and Amyloid-β fibrils. Left side representing 3D (k) and the right side representing 2D complex protein–ligand interaction (l).

3.6. MD Simulation Studies

Molecular dynamics simulation studies were used to investigate the stability of protein-ligand interactions. The protein-ligand complexes of amyloid-β fibrils-prenyl methoxy flavonol, amyloid-β fibrils-isopentenyl flavonol, amyloid-β fibrils-7,3'-Dihydroxyflavone, amyloid-β fibrils-7-Hydroxy-5-

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methyl-4'-methoxyflavone, and amyloid-β fibrils-8-hydroxy-7-methoxyflavone, as well as amyloid-β fibrils-donepezil complexes, were studied using MD simulations. The RMSD graph and ligand-protein interactions (2D interaction diagram) were examined to grasp the simulated results thoroughly. The RMSD graphs show the evolution of the protein (left y-axis) and its ligand (right y-axis). The amyloid-β fibrils-prenylmethoxy flavonol complex was stable, with protein root-mean-square deviation (RMSD) ranging from 4.8 to 7.1 Å and ligand RMSD ranging from 12.5 to 18.2 Å (Figure 3a). The MD trajectory events of the amyloid-β fibrils-isopentenyl flavonol complex showed that the protein RMSD was between 4.2 and 4.8 Å. In comparison, the ligand RMSD varied from 24 to 32 Å, indicating the stable complex (see Figure 3b). The MD trajectory events of the amyloid-β fibrils-7,3'-Dihydroxyflavone complex showed that the protein RMSD was between 8 and 20 Å, while the ligand RMSD ranged from 36 to 42 Å, showing the complex is non-stable (Figure 3c). The MD trajectory events of the amyloid-β fibrils-7-Hydroxy-5-methyl-4'-methoxyflavone complex showed that the protein RMSD was between 3.8 and 4.6 Å, while the ligand RMSD varied from 4 to 6 Å, indicating the stable complex (Figure 3d). The MD trajectory events of the amyloid-β fibrils-8-hydroxy-7-methoxyflavone complex showed that the protein RMSD was between 4 and 4.5 Å, while the ligand RMSD ranged from 10 to 14 Å, indicating a stable complex (Figure 3e). The MD trajectory events of the amyloid-β fibrils-donepezil combination showed that the protein RMSD was between 31 and 32 Å. In contrast, the ligand RMSD varied from 20 to 21 Å, indicating a highly stable complex (Figure 6(f)). The RMSF analysis of stable six protein-ligand complexes showed no significant changes when ligands were bound to the key functional groups of amyloid-β fibrils (Figures 3g–l). The amyloid-β fibrils- prenylmethoxy flavonol complex MD trajectory investigated the interactions of two specific amino acids (ALA30 and GLY29) with the protein. Based on the residue index, it was noted that these amino acids remained constant during the MD simulation (Figures 4a,b). The MD trajectory of the amyloid-β fibrils-isopentenyl flavonol complex revealed a stable link between two specific amino acids (ALA30 and GLY29), as demonstrated by their residue index and lack of volatility during simulation. The protein-ligand interactions of the amyloid-β fibrils-isopentenyl flavonol complex revealed that amino acid residues ALA30 and GLY29 had the most significant interaction with isopentenyl flavonol (Figures 4d,e). The protein-ligand interactions of the amyloid-β fibril-7,3'-Dihydroxyflavone complex revealed that amino acid residues 27B ASN, 27C ASN, 27D ASN, and 30D ALA contributed the most significant contact with 7,3'-Dihydroxyflavone (Figures 4g,h). The protein-ligand interactions of the amyloid-β fibrils-7-Hydroxy-5-methyl-4'-methoxyflavone complex revealed that amino acid residues 27D ASN and 30D ALA contributed the most significant interactions with 7-Hydroxy-5-methyl-4'-methoxyflavone (Figures 4j,k). The protein-ligand interactions of the amyloid-β fibril-8-hydroxy-7-methoxyflavone complex revealed that amino acid residues 27B ASN, 27C ASN, and 30C ALA had the most significant contact with 8-hydroxy-7-methoxyflavone (Figures 4m,n). The protein-ligand interactions of the amyloid-β fibrils-donepezil complex revealed that amino acid residues 30B ALA, 30D ALA, and 27D ASN had the most significant interaction with Donepezil (Figures 4p,q). Figures 4c,f,I,l,o,r) depict hydrogen bond interactions and a timeline of all amino acid residues that form H-bonds, hydrophobic, ionic, or water bridges. Darker lines imply a steady interaction with the object. These connections kept the protein-ligand combination stable throughout the molecular docking simulations. Based on our findings, in vitro studies with prenylmethoxy flavonol, isopentenyl flavonol, 7,3'-Dihydroxyflavone, 7-Hydroxy-5-methyl-4'-methoxyflavone, and 8-hydroxy-7-methoxyflavone should be done to determine their capacity to disaggregate amyloid-β fibrils. These compounds could be used as the cornerstone for future lead optimization.

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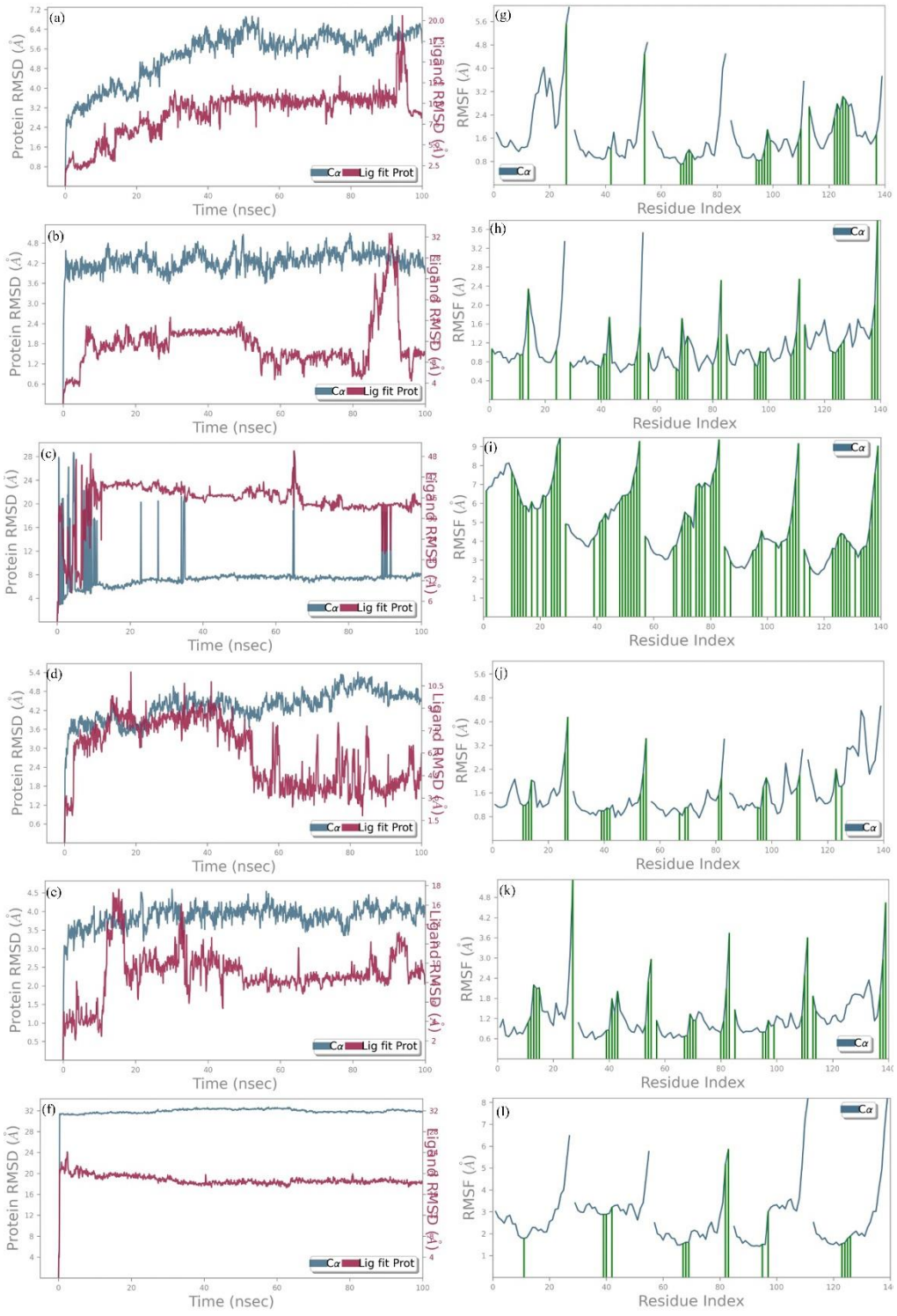

Figure 3. RMSD study plot for 100 ns MD simulation of prenylmethoxy flavonol-Amyloid-β fibrils docked complex (a), isopentenyl flavonol-Amyloid-β fibrils docked complex (b), 7,3'-Dihydroxyflavone-Amyloid-β fibrils docked complex (c), 7-Hydroxy-5-methyl-4'-methoxyflavone-Amyloid-β fibrils docked complex (d), 8-hydroxy-7-methoxyflavone-Amyloid-β fibrils docked complex (e), and Donepezil-Amyloid-β fibrils docked complex (f). Root mean square fluctuation (RMSF) of the Prenylmethoxy flavonol for characterizing changes in the ligand atom positions (a), root mean square fluctuation of the isopentenyl flavonol for characterizing changes in the ligand atom positions (b), root mean square fluctuation of the 7,3'-Dihydroxyflavone for characterizing changes in the ligand atom positions (c), root mean square fluctuation of the 7-Hydroxy-5-methyl-4'-methoxyflavone for characterizing changes in the ligand atom positions (d), root mean square

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fluctuation of the 8-hydroxy-7-methoxyflavone for characterizing changes in the ligand atom positions (e), and root mean square fluctuation of the Donepezil for characterizing changes in the ligand atom positions (f).

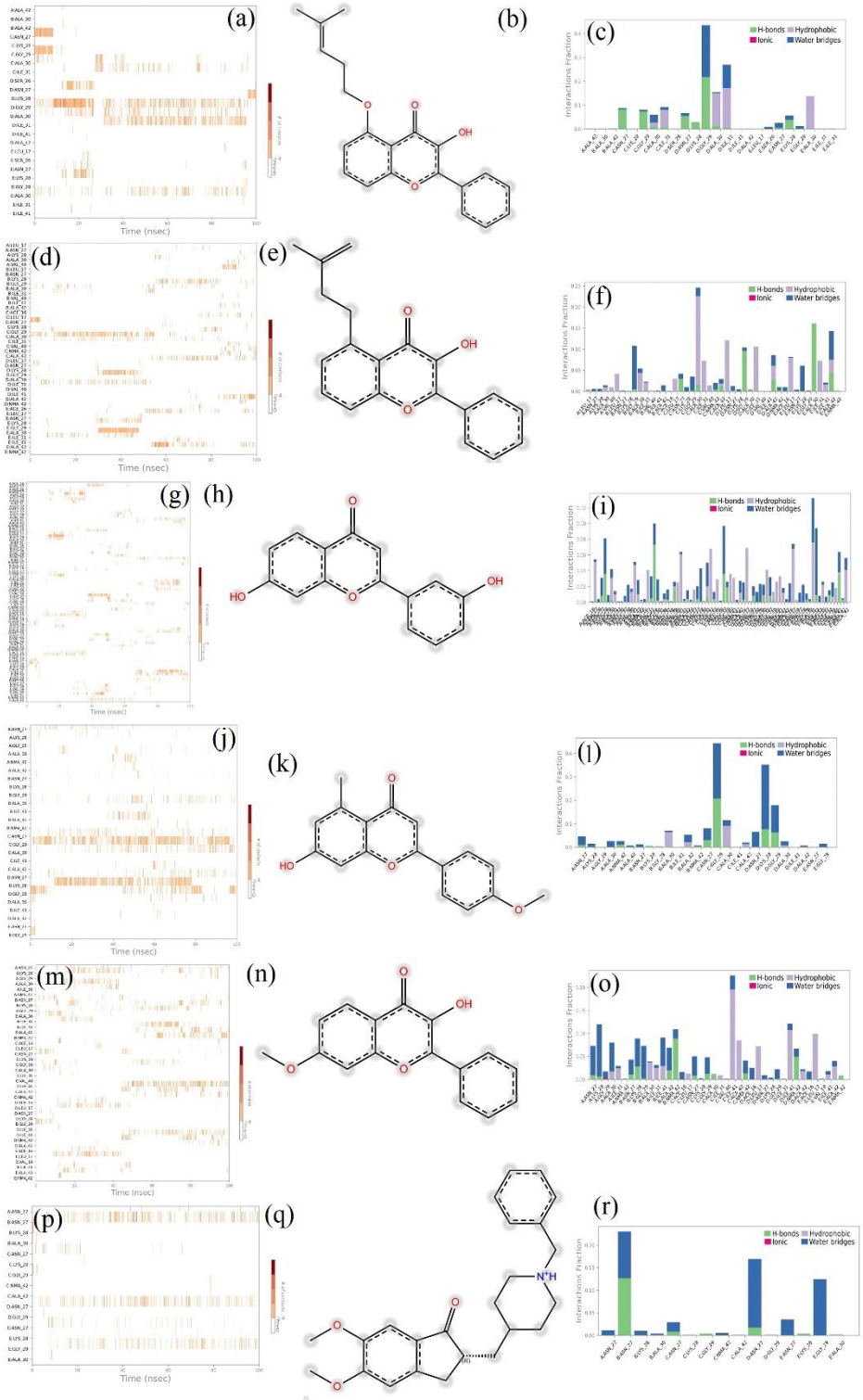

Figure 4. Prenylmethoxy flavonol-Amyloid-β fibrils docked complex timeline representation of the Prenylmethoxy flavonol (right side) (a), contacts with respect to the amino acids in the target (centre) (a). Percentage of amino acid and water-mediated interactions in MD simulations with Prenylmethoxy flavonol (left side) (c); Isopentenyl flavonol -Amyloid-β fibrils docked complex timeline representation of the isopentenyl flavonol (right side) (d), contacts with respect to the amino acids in the target (centre) (e). Percentage of amino acid and water-mediated interactions in MD

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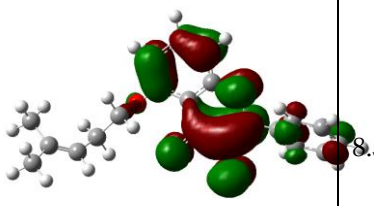
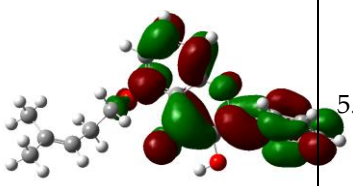
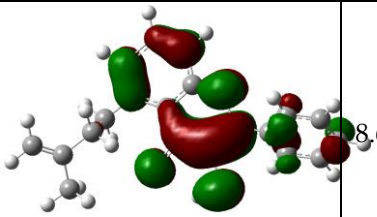
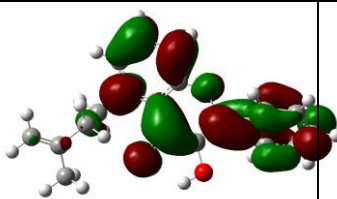
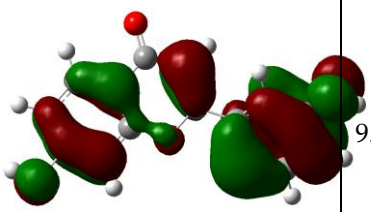
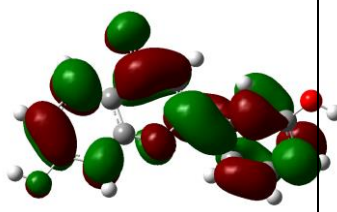
simulations with isopentenyl flavonol (left side) (f); 7,3'-Dihydroxyflavone-Amyloid-β fibrils docked complex timeline representation of the 7,3'-Dihydroxyflavone (right side) (g), contacts with respect to the amino acids in the target (centre) (h). Percentage of amino acid and water-mediated interactions in MD simulations with 7,3'-Dihydroxyflavone (left side) (i); 7-Hydroxy-5-methyl-4'-methoxyflavone-Amyloid-β fibrils docked complex timeline representation of the 7-Hydroxy-5-methyl-4'-methoxyflavone (right side) (j), contacts with respect to the amino acids in the target (centre) (k). Percentage of amino acid and water-mediated interactions in MD simulations with 7-Hydroxy-5-methyl-4'-methoxyflavone (left side) (l); 8-hydroxy-7-methoxyflavone-Amyloid-β fibrils docked complex timeline representation of the 8-hydroxy-7-methoxyflavone (right side) (m), contacts with respect to the amino acids in the target (centre) (n). Percentage of amino acid and water-mediated interactions in MD simulations with 8-hydroxy-7-methoxyflavone (left side) (o); Donepezil-Amyloid-β fibrils docked complex timeline representation of the Donepezil (right side) (p), contacts with respect to the amino acids in the target (centre) (q). Percentage of amino acid and water-mediated interactions in MD simulations with Donepezil (left side) (r).

3.7. DFT Studies

Frontier molecular orbitals (FMOs) are used to predict the chemical systems' most reactive regions and describe different reactions. In the sphere of material research, the FMO features—which include the HOMO and LUMO orbitals—are invaluable for characterizing materials. They are used to measure the molecules' chemical reactivity [33]. The energies of the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and the HOMO-LUMO energy gap were calculated at the B3LYP level using the 6-311 G (d, p) basis set. Table 1 showed the HOMO-LUMO images of the five highest-binding compounds in addition to the reference medication donepezil.

Table 1. EHOMO and ELUMO and Δev values of selected top binding scored compounds and standard drug Donepezil.

Compound Name	HOMO	EHOMO (ev)	LUMO	ELUMO (ev)	Energy gap (Δev)
Prenylmethoxyflavonol		-8.578		-5.828	2.750
Isopentenyl flavonol		-8.604		-5.917	2.687
7,3'-Dihydroxyflavone		-9.285		-5.880	3.404

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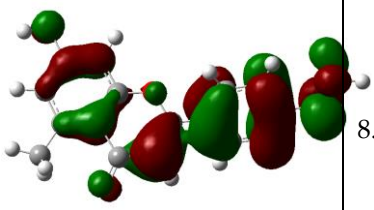
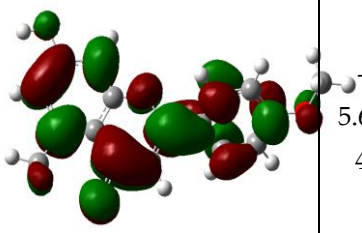
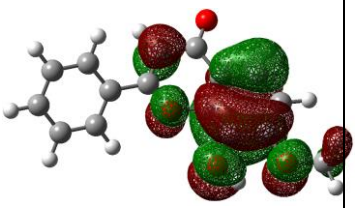
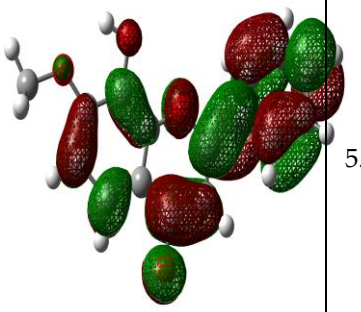
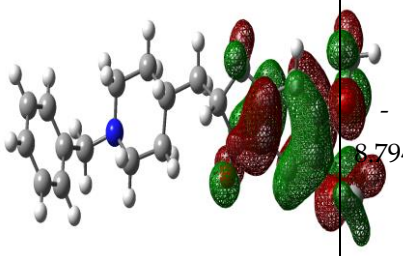
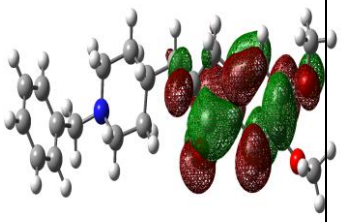
7-Hydroxy-5-methyl-4'-methoxyflavone		-8.771		-5.654	3.117
8-hydroxy-7-methoxyflavone		-8.515		-5.850	2.665
Donepezil		-8.794		-5.667	3.127

4. Conclusion

Traditionally, plant extracts and their secondary metabolites have exhibited numerous chemical constituents that aid in preventing various diseases. These molecules possess high levels of security, exhibit low toxicity levels, and demonstrate economic efficiency, thus enhancing the overall quality of human existence. The aggregation of amyloid-β fibrils in the brain is a characteristic feature of Alzheimer's disease. This work has found five potential molecules, namely prenylmethoxy flavonol, isopentenyl flavonol, 7,3'-Dihydroxyflavone, 7-Hydroxy-5-methyl-4'-methoxyflavone, and 8-hydroxy-7-methoxyflavone, that can disaggregate amyloid-β fibrils. Molecular docking experiments identified these molecules. MD simulation assessed the ligand-receptor complex's stability, revealing that all five Aβ oligomer protein complexes remained stable throughout the simulation. The results obtained from density functional theory also indicated that all five compounds exhibit high stability. In conclusion, the five discovered molecules have the potential to serve as lead compounds for further testing as potential neuroprotective agents that can act on Aβ fibril disaggregation. These compounds can be evaluated utilizing both *in vitro* and *in vivo* experiments.

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