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Confirmation of the Experimentally-Proven Therapeutic Utility of Madecassoside in an A$\beta_{1\text{-}42}$ Infusion Rat Model of Alzheimer's Disease by *in Silico* Analyses

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Abstract

The accumulation of amyloid β peptide 1 - 42 (A$\beta_{1\text{-}42}$) in the brain of Alzheimer's disease (AD) patients is known to be associated with neurodegeneration and memory impairment. More recently, we reported that madecassoside, an active component of *Centella asiatica*, improved memory impairment in an A$\beta_{1\text{-}42}$ infusion rat model of AD, ameliorated neurotoxicity in SH-SY5Y cells, and inhibited *in vitro* A$\beta_{1\text{-}42}$ fibril formation. In the present study, we investigated the utility of *in silico* analyses in corroborating observed *in vivo* and *in vitro* effects of madecassoside in AD to further assess the therapeutic benefits of madecassoside. The 3D structure of A$\beta_{1\text{-}42}$ was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). The binding of madecassoside to A$\beta_{1\text{-}42}$ was assessed by molecular docking. The chemical structure of madecassoside was modeled and converted to the PDB format. Madecassoside was found to successfully dock with A$\beta_{1\text{-}42}$. Computational demonstration of the binding of madecassoside to A$\beta_{1\text{-}42}$ further corroborated the inhibitory effect of madecassoside on A$\beta_{1\text{-}42}$ fibrillogenesis which was demonstrated in our previous study. These data showed the potential utility of madecassoside as a preventive medication in A$\beta_{1\text{-}42}$-induced neurodegenerative diseases such as AD.

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Keywords

Alzheimer's Disease, *Centella asiatica*, Madecassoside, Ayurveda, *in Silico*, Drug Designing

1. Introduction

Alzheimer's disease (AD) is a dementia-related neurodegenerative disease. AD is pathologically characterized by fibrillar deposition of amyloid β (Aβ) peptides in the brain [1]. The A$\beta_{1\text{-}42}$ peptide is the predominant constituent of deposits observed in the affected brain of AD patients [2]. The prevalence of AD is 10% in 65-year-old people, and it increases to 50% in individuals aged over 80 years old, indicating an increasing prevalence of AD in elderly people. Alzheimer's disease International (ADI) estimates that there are currently 30 million people with dementia worldwide, with 4.6 million new cases annually (one new case every 7 s). The number of people affected is estimated to reach more than 100 million by 2050 [3]. AD is the fourth leading cause of death in individuals over the age of 65. AD is a progressive neurodegenerative disorder with insidious onset characterized by severe decline in episodic memory.

Madecassoside, a highly polyphenolic compound, is the major triterpene glycoside found in *Centella asiatica* [4]. We previously reported that purified madecassoside inhibits *in vitro* A$\beta_{1\text{-}42}$ fibril formation [5]. More recently, we reported that long-term oral administration of purified madecassoside protected against spatial memory impairment in A$\beta_{1\text{-}42}$-infused AD model rats and inhibited A$\beta_{1\text{-}42}$ fibril formation, as indicated by ThioflavinT (ThT) fluorometry, laser scanning microscopy, and transmission electron microscopy [6]. We further demonstrated that co-treatment with madecassoside significantly attenuated A$\beta_{1\text{-}42}$-induced apoptosis in SH-SY5Y cells, with concurrent decreases in the levels of lipid peroxidation, A$\beta_{1\text{-}42}$ burden, and TNF-α and increases in hippocampal levels of Brain derived neurotrophic factor (BDNF) and postsynaptic density protein (PSD-95) [6]. Our results clearly indicated that madecassoside provided therapeutic benefits in AD. Despite a lack of clarification regarding the interaction between madecassoside and A$\beta_{1\text{-}42}$, agents capable of targeting amyloid toxicity leading to memory impairment in AD have been extensively researched. Although we previously demonstrate the therapeutic utility of madecassoside in animal models, the exact mechanismofaction of madecassoside is yet to be determined. Therefore in this study, we developed models of the binding of madecassoside, as the ligand, to A$\beta_{1\text{-}42}$, as the receptor, to further evaluate the anti-A$\beta_{1\text{-}42}$ fibrillation effect of madecassoside demonstrated in our previous study [6].

Structure (target)-based drug design by molecular docking represents an interesting venue to identify and optimize drug candidates by examining and modeling molecular interactions between ligands and target macromolecules. The fluorescent dye Thioflavin-T (ThT) has become among the most widely used "gold standards" for selectively binding, staining and identifying amyloid fibrils both *in vivo* and *in vitro*. The large enhancement of its fluorescence emission upon binding to amyloid fibers makes ThT a particularly powerful and convenient tool. For the extraordinary ability of ThT to recognize and bind with the amyloid fibrils, here, we used the ThT-fibril interactions as positive control. We docked madecassoside as ligand to the A$\beta_{1\text{-}42}$, as receptors. We analyzed the binding of madecassoside in the context of the interaction of ThT with A$\beta_{1\text{-}42}$.

2. Methods

2.1. *In Silico* Studies

The software/web servers used were Marvin Sketch, Molegro Virtual Docker, Patch Dock, and Rossetta Dock.

2.2. Preparation of Docking Materials: ThT and Madecassoside (Ligands) and A$\beta_{1\text{-}42}$ Dimers and Trimers (Receptors)

The canonical Simplified Molecular Input Line Entry System (SMILES) strings of ThT (Chemical Identification number: 16,953; **Figure 1**) and madecassoside (Chemical Identification number: 45,356,919; **Figure 1**) were submitted to Marvin 5.7.0 [7] to generate the three-dimensional (3D) structure of each ligand molecule. The 3D structures of ThT and madecassoside were subsequently energy-minimized and converted to the Protein Data Bank (PDB) file format using the Molegro Virtual Docker (MVD) [8]. A$\beta_{1\text{-}42}$ (PDB ID: 2BEG) was downloaded

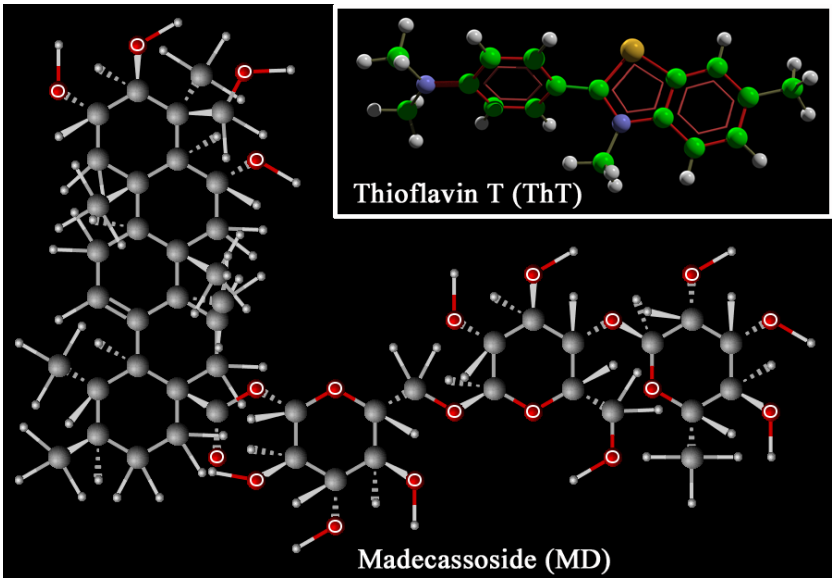

Figure 1. The ligand molecules: thioflavin T (ThT) and madecassoside (MD).

from the PDB as a receptor for ThT and madecassoside docking. 2BEG is a 3D nuclear magnetic resonance solution structure of A$\beta_{1\text{-}42}$, comprising residues 18 - 42 of a β-strand-turn-β-strand motif, and contains two intermolecular β-sheets that are formed by residues 18 - 26 (β 1) and 31 - 42 (β 2). 2BEG is a homopentamer, namely composed of A, B, C, D, and E monomer of A$\beta_{1\text{-}42}$. Each monomer of 2BEG comprises 10 coordinate models [9]. The coordinates of model 1 of A monomer (A1), model 1 of B monomer (B1), and model 1 of C monomer (C1) were split from the composite 2BEG PDB file by MVD. Subsequently, the dimer, A1B1, was generated by Rossetta Dock [10] based on energy minimization. A trimer, A1B1C1, was also generated for madecassoside docking.

2.3. Molecular Docking of ThT and Madecassoside onto A$\beta_{1\text{-}42}$ Dimer and Trimer

2.3.1. Docking with MVD

The docking simulation was performed using the docking software MVD [8]. MVD is an automated docking software program with fast processing that automatically adds the missing hydrogen atoms of the ligand and receptor molecules, if any. The software also has a module to create a surface over the receptor molecule and identify potential binding sites for its activity. The program gives 10 conformational positions or so-called poses for the ligand and returns the five best poses with Mole Dock Score (equivalent to energy of binding/docking energy) and other thermodynamically calculated values. MVD also presents hydrogen bond information together with other thermodynamic values that suggest the formation of stable complexes between ligands and receptor molecules. MVD performs flexible ligand docking with optimization of the ligand geometry during docking.

2.3.2. Docking with Patch Dock

Patch Dock [11] is a geometry-based molecular docking algorithm designed to find docking transformations that yield good molecular shape complementarity. Such transformations, when applied, induce both wide interface areas and small amounts of steric clashes. A wide interface ensures the inclusion of several matched local features of docked molecules with complementary characteristics.

3. Results

3.1. Molegro Virtual Docker (MVD)

Docking performed by MVD provided the five best poses with corresponding Mol Dock score values and other thermodynamically calculated values. The 3D structures of bests coring dockings are shown in **Figures 2(a)-(c)**. Steric interactions of both ThT and madecassoside, as ligands, with A$\beta_{1\text{-}42}$ (the receptor) were also evaluated

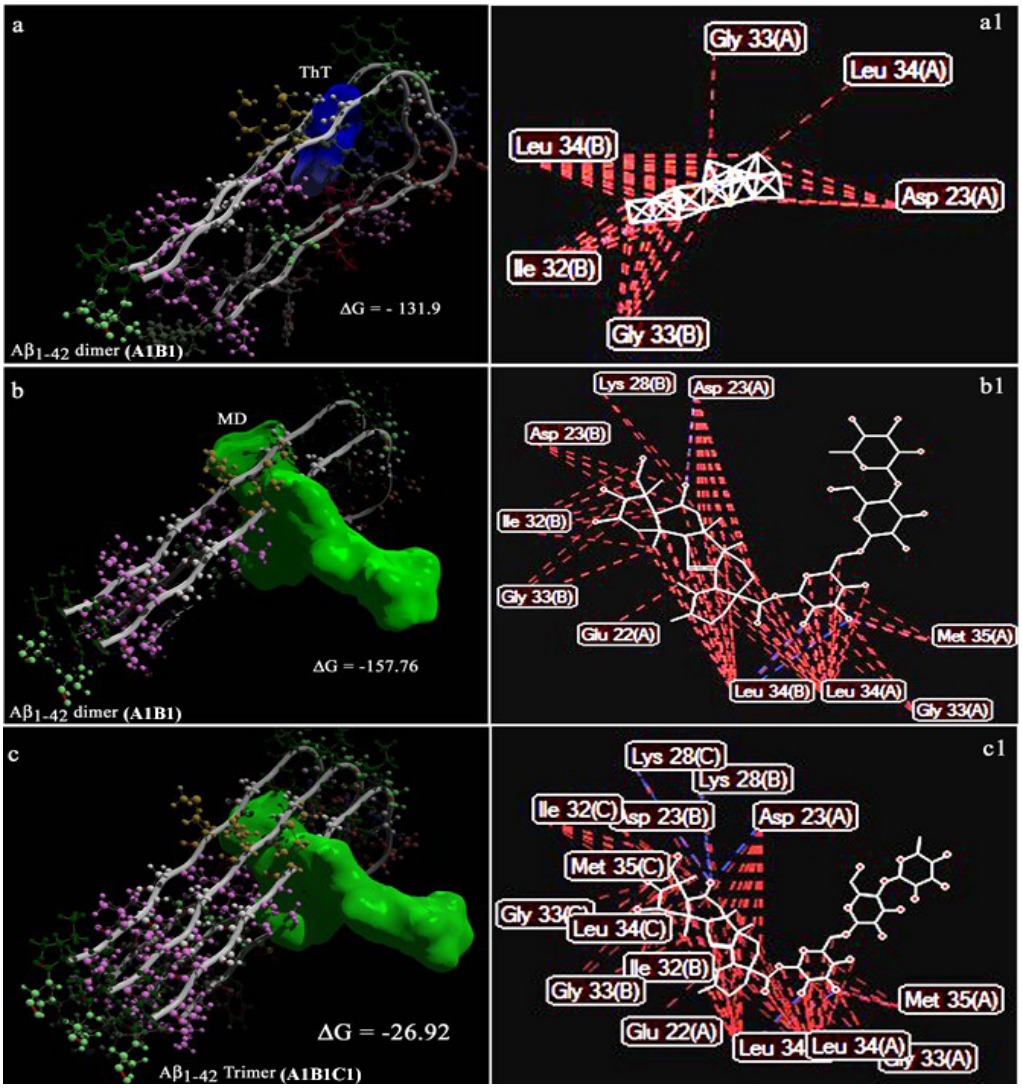

Figure 2. The best docking poses of the ligands, thioflavin T (ThT) and madecasoside (MD) with the receptors (A$\beta_{1\text{-}42}$). ThT in pose 1 docked onto the A1B1 dimer (a), MD in pose 1 docked on toA1B1 dimer (b) and A1B1C1 trimer (c). Each monomeric strand, consisting of in-register parallel β1- and β2-sheets, runs perpendicularly along the long axis of the fiber. Interaction (binding) energies (−ΔG), shown as MolDock scores, are shown in (a)-(c). (a1)-(c1): contact (binding) maps of the interactions between atoms of ThT/MD and amino acid residues of the dimer/trimer (see also **Table 1**). Contact maps were visualized by Molegro Virtual Docker (MVD). Blue lines, formation of hydrogen bonds; red lines, steric interactions. A1, B1, and C1 are the corresponding coordinates of model 1 for the A, B, and C monomers of A$\beta_{1\text{-}42}$, respectively.

with MVD. Maps of the interactions of ThT with amino acid residues of the A$\beta_{1\text{-}42}$ dimer (A1B1) are shown in **Figure 2(a1)**. The docking of madecassoside to trimeric A$\beta_{1\text{-}42}$ (A1B1C1) and its interaction map are shown in **Figure 2(c1)**. The binding of madecassoside to the dimer was stronger than that of the trimer, as demonstrated by decreased binding energies (**Figures 2(b)-(c)**). Amino acids involved in the docking of madecassoside to the dimer (A1B1) and trimer (A1B1C1) were visualized by contact maps of the interactions between atoms of madecassoside and the amyloid amino acid residues using MVD (**Figures 2(b1)-(c1)**, and **Table 1**). The amino acid residues of the A1B1 dimer found to sterically interact with ThT were Asp23, Gly33, and Leu34 of the A1 monomer and Ile32, Gly33, and Leu34 of the B1 monomer (**Figures 2(a)-(a1)**). Madecassoside sterically interacted with Glu22, Asp23, Gly33, Leu34 and Met35 of the A1 monomer, and Asp23, Lys28, Ile32, Gly33, Leu34 of the B1 monomer. These results clearly indicate that ThT and madecassoside have common binding sites at

Table 1. Interaction sites of thioflavin T (ThT) and madecassoside (MD) with the amino acid residues of amyloid dimer (A1B1) and trimmer (A1B1C1).

Amino acid residues			Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys	Gly	Ala	Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala
	1 - 20		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
ThT	Dimer	A1																						
		B1																						
MD	Dimer	A1																						
		B1																						
	Trimer	A1																						
		B1																						
		C1																						

Asp23 of the β1-sheet and Gly33 and Leu34 of the β2-sheet of the A1 monomer. These two ligands also had identical binding sites for the B1 monomer. In addition, the higher free energy of binding of madecassoside than ThT indicates stronger binding of madecassoside to the dimer (A1B1). Madecassoside was also found to bind with the amyloid trimer (A1B1C1); however, the binding energy was lower than that observed for the binding of madecassoside to the dimer (A1B1).

3.2. Patch Docker

After performing molecular docking with MVD, we performed molecular docking of ThT and madecassoside using Patch Dock to confirm the ability of madecassoside to bind amyloid A1B1 dimer and A1B1C1 trimer (**Figure 3**). The algorithm used in Patch Dock performs rigid docking, with surface variability/flexibility implicitly addressed through liberal intermolecular penetration. Geometric shape complementarity scores were higher for madecassoside than for ThT when docked with both the dimer and the trimer. Approximate complex interface areas (receptor-ligand) and atomic contact energies were also higher for madecassoside (**Table 2**), suggesting stronger binding of madecassoside to the A1B1 dimer and A1B1C1 trimer than that of ThT.

4. Discussion

A$\beta_{1\text{-}42}$ peptides are important drug development targets because of their crucial role in neuronal diseases, including AD. Thus, the design of drugs that disrupt the formation of A$\beta_{1\text{-}42}$-A$\beta_{1\text{-}42}$ interfaces is particularly important for the development of therapies for the inhibition of A$\beta_{1\text{-}42}$ fiber formation and/or the dismantling of preformed A$\beta_{1\text{-}42}$ fibers. In other words, the development of A$\beta_{1\text{-}42}$ fibrillation antagonists may provide an increased understanding of the pathogenesis of AD, leading to the development of more effective therapies. We previously demonstrated that co-incubation of free A$\beta_{1\text{-}42}$ monomers with madecassoside prevented fiber polymerization [5]. The inhibitory effect of madecassoside on A$\beta_{1\text{-}42}$ fibril formation corroborated previous ThT fluorescence spectroscopy and transmission electron microscopy findings [6]. In this study, our intention was to computationally model the interaction of madecassoside with A$\beta_{1\text{-}42}$ by molecular docking. Ligand binding sites for A$\beta_{1\text{-}42}$ were examined using high-resolution 3D coordinates obtained from the PDB. Finally, we sought to obtain information regarding the mechanism of madecassoside-induced inhibition of amyloid fibril formation by computationally modeling the binding of ThT with A$\beta_{1\text{-}42}$. We previously reported that Aβ fibers bind to ThT [12]-[15]. ThT is one of the most widely used amyloid dyes used in the hundreds of amyloid studies published every year. The intensive use of ThT as an *in vitro* marker of amyloid formation has provided substantial information regarding the mechanism of ThT binding. Further studies aim to fully determine common structure(s) of the amyloid peptide recognized by ThT. Within the past several years, a series of critical experimental analyses of ThT have elucidated, in detail, many of the atomiclevel interactions underlying ThT binding and fluorescence. Because several recent reports have discussed the broad range of amyloid staining dyes [16]-[18], in the present study, we instead focused on the atomiclevel mechanisms underlying the binding of ThT to amyloid fibrils. Amyloid fibrils may have a specific ThT binding site that sterically "locks" the bound dye, leading to the enhancement of ThT fluorescence. These properties of ThT made it an ideal positive reference compound for this

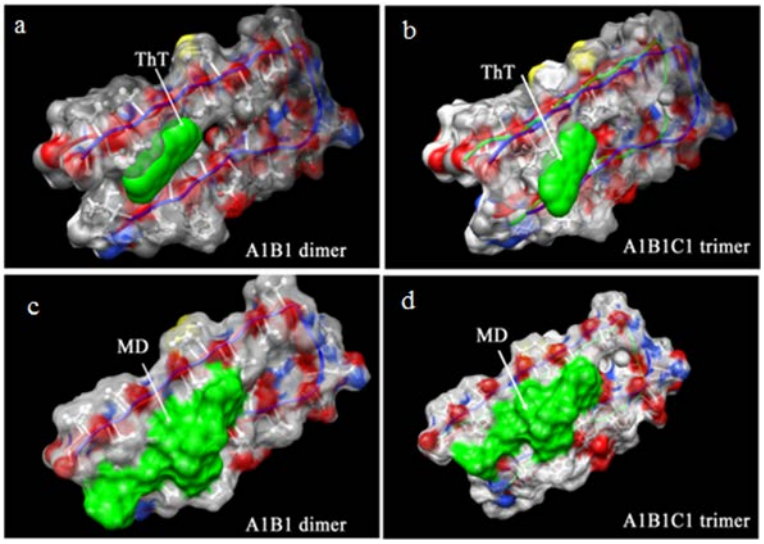

Figure 3. The best scoring results of the Patch Docking of Thioflavin T (ThT) (a)-(b) and madecassoside (MD) (c)-(d) with theA1B1 dimer and A1B1C1 trimer. Ligands (ThT and MD) are represented by green surface-fill representation and the receptors (A1B1 dimer and A1B1C1 trimer) are in ribbons + surface presentation.

Table 2. Patch docking of thioflavin T (ThT) and madecassoside (MD) with the amyloid dimer (A1B1) and trimer (A1B1C1).

Docking (Receptor vs. Ligands)	Score	Area	ACE
A1B1 dimer vs. ThT	2942	356.20	−314.68
A1B1 dimer vs. MD	6130	789.50	−470.03
A1B1C1 trimer vs. ThT	2820	307.50	−261.45
A1B1C1 trimer vs. MD	6586	845.50	−460.37

Score: Geometric shape complementarity score; Area: Approximate interface area of the complex (receptor-ligand); ACE: Atomic contact energy; A1B1 dimer: Generated by feeding the A1 and B1 model 1 of 2BEG to Rosetta Server; A1B1C1 trimer: Generated feeding the A1, B1 and C1 coordinate model 1 of 2BEG; ThT:Thioflavin T and MD: madecassoside.

study.

Structure-based drug design has made substantial contributions to drug discovery, including the development of treatments for cancer and drug-resistant infectious diseases. Computational structure-based drug design may, therefore, facilitate the development of novel treatments for AD. We recently reported that asiaticoside, another polyphenol compound of *Centella asiatica*, also inhibited fibril formation [19]. Therefore, the docking of madecassoside with the A$\beta_{1\text{-}42}$ dimer/trimer was used to corroborate our previous findings regarding the *in vivo* and *in vitro* inhibitory effects of madecassoside on fibril formation and memory impairment in an AD rat model [6]. Aggregation-prone amino acids, *i.e*., hotspots for A$\beta_{1\text{-}42}$ aggregation, are located at residues 17 - 22 and 32 - 42 [19].

While docked with the dimer/trimer, madecassoside displayed greater binding affinity, according to binding energy, than ThT. These results demonstrate that madecassoside is capable of binding amyloid fibers, potentially providing an explanation for the reduced amyloid burden observed in the brains of madecassoside-administered AD model rats and concurrent inhibition of *in vitro* fibril formation [6]. Notably, residues 17 - 21 and residues 31 - 42 are β-sheet forming amino acids engaged in monomer-monomer (dimer) inter surface interactions [19]. The common features between MVD and Patch Docke were displayed in their common amino acid binding sites for madecassoside: Asp23 and Leu34 of the A$\beta_{1\text{-}42}$ (analysis not shown). These results again suggest that inter surface interaction sites is the most promising target sites for ligands that potentially inhibit amyloid fibril interactions, such as madecassoside. Hossain *et al.* (2009) previously reported that the dimeric/trimeric form of A$\beta_{1\text{-}42}$ acts as one of the seeding units for A$\beta_{1\text{-}42}$ fibril formation [12]. Therefore, we assessed the molecular docking of the dimeric/trimeric structures of A$\beta_{1\text{-}42}$ with madecassoside. Polyphenols are capable of forming hydrogen bonds with amyloids and other proteins [20] [21]. In the present *in silico* study, madecassoside was

found to affect A$\beta_{1\text{-}42}$ fibril formation through binding to amino acid residues 17 - 21 and 31 - 42, the most aggregation-sensitive regions of A$\beta_{1\text{-}42}$, and forming hydrogen bonds with A$\beta_{1\text{-}42}$. The neurotoxicity of A$\beta_{1\text{-}42}$ involves the *in vivo* conformational transition from soluble α-helical to insoluble β-sheet forms of the peptide following release from the cell membrane into the surrounding aqueous environment.

5. Conclusion

Herein, we demonstrate the successful molecular docking of madecassoside on to A$\beta_{1\text{-}42}$, with a higher affinity of binding than that with the thioflavin T, consistent with the inhibitory effects of madecassoside on *in vitro* fibril formation and memory impairment in a rat model of AD. Our *in silico* results further provide compelling evidence for the utility of triterpene glycosides as a preventive medication for neurodegenerative diseases such as A$\beta_{1\text{-}42}$ aggregation-induced AD.

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List of Abbreviations

AD: Alzheimer's Disease;
RCSB: Research Collaboratory for Structural Bioinformatics;
PBD: Protein Data Bank;
MVD: Molegro Virtual Docker;
ThT: Thioflavin-T;
BDNF: Brain derived neurotrophic factor;
PSD-95: Postsynaptic density protein-95;
MD: Madecassoside;
SMILES: Simplified Molecular Input Line Entry System.