Presented at the DLSU Research Congress 2025
De La Salle University, Manila, Philippines
June 27, 2025

Exploring Potential Molecular Targets of *Ginkgo biloba* Related to Alzheimer's Disease Pathology

Michael Josh Ramirez[1,2], Lyka Porras [1,2], Marion Jacildo[1,2] and Jane Abigail Santiago[1,2]
[1] *De La Salle University*
[2] *College of Science, Department of Biology*
**Corresponding Author: michael_ramirez@dlsu.edu.ph*

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive decline and accumulation of amyloid-beta plaques and tau tangles in the brain, alongside neuroinflammation and oxidative stress. Current treatments are limited, necessitating alternative therapeutic approaches. *Ginkgo biloba*, with its antioxidant and anti-inflammatory compounds, shows potential in targeting AD pathology. This study investigated molecular interactions between *Ginkgo biloba* compounds and key AD-related proteins, including neurolysin, serum albumin, thyroid hormone, and matrix metalloproteinase-3, using molecular docking and ADME (Absorption, Distribution, Metabolism, and Excretion) screening. The objectives were to confirm interactions and determine the compounds' anti-AD potential based on blood-brain barrier (BBB) permeability, binding affinity, oral bioavailability (OB), and drug-likeness (DL) values. This study underscored a prioritization framework based on BBB, binding affinity, OB, and DL, essential for CNS-targeted treatments. Five compounds showed the most promising anti-AD potential, particularly genkwanin, ethyl oleate, stigmasterol, beta-sitosterol, and mandenol, due to favorable BBB permeability and binding affinity, for targets such as neprilysin, serum albumin, and thyroid hormone. Findings support the potential of *Ginkgo biloba* compounds for AD therapy, laying a foundation for further experimental validation.

Key Words: Alzheimer's Disease (AD), *Ginkgo biloba*, Molecular Docking, Blood-Brain Barrier (BBB) permeability, Anti-AD Potential

1. INTRODUCTION

Alzheimer's disease (AD) is one of the neurodegenerative disorders characterized by cognitive decline, memory impairment, and buildup of amyloid-beta (Aβ) plaques and tangles of tau protein in the brain (Tahir et al., 2024). These pathological hallmarks, alongside neuroinflammation and oxidative stress contribute to irreversible brain damage as seen in AD patients (Willette et al., 2022). Despite significant advances in understanding AD's pathology, current therapeutic approaches are limited to the management of symptoms, leaving an urgent need for interventions that target the underlying mechanisms of the disease (Huang et al., 2016).

Natural compounds, especially those extracted from plants like *Ginkgo biloba* have been long used in traditional medicine, and its bioactive compounds such as ginkgolides and bilobalide, which exhibit properties that could target mechanisms underlying AD's pathology. These compounds are known for their antioxidant, anti-inflammatory, and anti-apoptotic properties making them promising for further research as potential AD therapeutics (Yang et al., 2016). However, the exact molecular interactions between these compounds and specific AD-related proteins are still unknown.

The purpose of this study is to address these

gaps by confirming interactions between bioactive compounds of *Ginkgo biloba* and putative protein targets involved in AD. Specifically, proteins such as neurolysin, serum albumin, thyroid hormone, and matrix metalloproteinase 3, have been implicated in AD pathology. Neurolysin, for instance, has been associated with amyloid-beta degradation (Nasb et al., 2024), while serum albumin has shown potential in inhibiting amyloid-beta aggregation (Kim et al., 2020). Using molecular docking simulations, the researchers evaluated the binding affinity of *Ginkgo biloba* compounds to these proteins, with a focus on identifying the strongest interactions. Molecular docking is a powerful tool that is often used in nutraceutics, a field that heavily focuses on researching the bioactive compounds present in food sources that contain therapeutic properties (Agu et al., 2023). Such a tool provides a way to determine the interactions of receptor proteins and these bioactive compounds, which could lead to the development of drugs, and ultimately, the management of diseases.

To complement molecular docking, absorption, distribution, metabolism, and excretion (ADME) screening was conducted to assess the pharmacokinetics and drug-likeness of compounds. This combined approach determined which Ginkgo biloba compound has the strongest therapeutic potential, not only based on binding affinity but also its sustainability as a drug candidate.

The Alzheimer's Society (2024) mentions that 55 million people are affected by dementia worldwide. Besides this, it is believed that by 2050, 139 million people will be affected by this disease. Despite the rising number of people with AD, this disease, along with other forms of dementia, does not have any cure. Additionally, the available drugs for AD could only slow down its development (Peng et al., 2023). These medicines include cholinesterase inhibitors and memantine (Namenda) helping in cell communication in the brain. While these medicines are able to help with the symptoms, these medicines also have side effects such as diarrhea, nausea, loss of appetite, sleep disturbances, dizziness, confusion, and more. Because of this, alternative medicines have been and are currently being studied to prevent AD and boost cognitive health. These include vitamin E, omega-3 fatty acids, curcumin, ginkgo, and melatonin. However, the Mayo Clinic states that the funded study by the National Institutes of Health found that ginkgo does not have any effect on the prevention of AD. Despite this, the amelioration of ginkgo is poorly studied.

Thus, the objectives of the study are to confirm the interaction between the putative protein targets of Alzheimer's Disease and the docking compounds of Ginkgo biloba and to determine which among the docking compounds of Neprilysin, Serum albumin, Thyroid hormone, and Matrix metalloproteinase 3 have the highest anti-AD potential by ranking them based on their ADME profile and binding affinity.

2. METHODOLOGY

2.1 Retrieval of Docking Compounds and Their Corresponding Protein Targets

The docking compounds, specifically (+)-Catechin, Diosmetin, Genkwanin, Ginkgolide B, Ginkgolide J, Ethyl oleate, Flavoxanthin, Beta-sitosterol, Stigmasterol, and Mandenol were collected from The Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (https://old.tcmsp-e.com/index.php). Their bioavailability (OB), blood-brain barrier (BBB), and drug-likeness (DL) values were recorded. The researchers retrieved the information on the six putative target proteins, specifically Neprilysin, Estrogen receptor, Prothrombin, Serum albumin, Thyroid hormone, and Matrix metalloproteinase from UniProt. A software called PyRx - Virtual Screening Tool (Python Prescription 0.8) was used to perform molecular docking, whereas the preparation of the putative protein targets that comprised the process of removing water molecules was performed in Discovery Studio 2024 Client.

2.2 Determination of Ligand-Binding Affinity

The researchers recorded the Binding Affinity Scores from the molecular docking results, along with the ADME profile that was retrieved from the TCSMP. The criteria will be OB 30%, DL 0.18, and BBB -0.3, as recommended by Li et al. (2018).

2.3 Prioritization Framework for Anti-AD Potential

To determine which phytochemical had the highest anti-AD potential with the putative target proteins, the researchers accounted for compounds with the most favorable docking score and ADME profile. A prioritization framework was employed, ranking the compounds' anti-AD potential based on Blood-brain Barrier (BBB), Binding Affinity (BA), Oral Bioavailability (OB), and Drug-likeness values (DL).

The rationale behind this order is that BBB permeability determines whether a compound can reach the CNS, making it the most crucial parameter for neurodegenerative diseases like AD (Daraban et al., 2024). BBB is a highly selective barrier that restricts the passage of most substances into the brain. Compounds with high BBB value are more likely to reach therapeutic

concentrations in the CNS, which is crucial for treating diseases that affect the brain (Pardridge et al., 2020). Without sufficient BBB penetration, even compounds with high binding affinity would not be effective in the brain.

Following BBB permeability, binding affinity is crucial as it indicates the strength of interactions between a compound and its target protein. High binding affinity indicates a stable and effective interaction, which is essential for modulating the target protein's function (Wang et al., 2019).

When the threshold of the first two criteria was met, researchers examined the oral bioavailability (OB) of the compounds. Oral bioavailability (OB) refers to the proportion of the drug that enters the systemic circulation and becomes available at the intended site, administered via the oral route (Price & Patel, 2023). A high OB value suggests that a significant portion of the drug is absorbed after administration, which is necessary for determining the drug dosage and maintaining therapeutic levels in the bloodstream (Stielow et al., 2023).

Least priority in the framework was drug-likeness. Drug-likeness (DL) measures how closely a compound's properties align with those of known drugs. It is based on factors like molecular weight, lipophilicity, and hydrogen bond donors or acceptors, that collectively influence a compound's pharmacokinetics and pharmacodynamics (Lipinski et al., 2012). DL helps identify compounds with properties similar to existing drugs, making it a valuable early-stage assessment tool. DL values were used as filters to screen compounds that may be difficult to develop into drugs but were less crucial once a compound met the BBB and binding affinity criteria. A compound with a high DL value is likely to have favorable absorption, distribution, metabolism, and excretion (ADME) properties.

Thus, the analysis ranking of BBB, binding affinity, OB, and DL ensured that the selected compounds are not only capable of reaching the brain but also have a strong interaction with their target proteins, followed by systemic bioavailability considerations and general drug-like properties. This order optimized the selection of potential drug candidates for CNS disorders and enhanced the likelihood of successful therapeutic outcomes.

3. RESULTS AND DISCUSSION

Table 1 shows the values obtained for the OB, DL, and BBB of the docking compound. Additionally, it shows their binding affinity towards the respective putative protein target. However, the putative protein targets prothrombin and estrogen receptor only has one docking compound. The target protein is said to have a better affinity with the docking compound if the binding affinity value is more negative. Thus, Neprilysin has a strong binding affinity with (+)-Catechin and Diomestin because the score is -8.3. On the other hand, serum albumin has a stronger affinity towards flavoxanthin, the thyroid hormone with Beta-sitosterol and Stigmasterol, and matrix metalloproteinase with Ginkgolide B.

Table 1. Bioavailability, Drug-likeness, and Blood-brain Barrier Values of the Docking Compound

Putative Protein Target	Docking Compound	BBB Values ≥ -0.3	Binding Affinity	OB Values ≥ 30%	DL Values ≥ 0.18
Neprilysin (NEP)	Catechin	-0.73	-8.3	54.83%	0.24
	Diosmetin	-0.66	-8.3	31.14%	0.27
	Genkwanin	-0.24	-7.7	37.13%	0.24
Estrogen receptor	Genkwanin	-0.24	-7.2	37.13%	0.24
Prothrombin	Ginkgolide J	-1.15	-9.8	44.84%	0.74
Serum Albumin (ALB)	Ethyl oleate	1.10	-5.7	32.40%	0.19
	Flavoxanthin	-0.90	-0.90	60.41%	0.56
Thyroid hormone (TTR)	Beta-sitosterol	1.11	-6.8	40.39%	0.85
	Stigmasterol	1.00	-6.8	43.83%	0.76
	Mandenol	1.14	-4.6	42.00%	0.19
	Ethyl oleate	1.10	-6.7	32.40%	0.19

	Flavoxanthin	-0.90	-7.3	60.41%	0.56
Matrix Metalloproteinase 3	Ginkgolide B	-1.56	-8.3	44.38%	0.73
	Ginkgolide J	-1.15	-7.9	44.84%	0.74

Among the six putative protein targets, four had multiple docking compounds, specifically Neprilysin (NEP), Serum Albumin (ALB), Thyroid Hormone (TTR), and Matrix Metalloproteinase (MMP3). The docking compounds were ranked based on the previously elucidated criteria (Table 2). Among the three compounds that target Neprilysin (NEP), genkwanin had the highest anti-AD potential because it is the only phytochemical that has a BBB value greater than or equal to -0.3. This signifies that the compound is able to pass through the blood-brain barrier, thereby possessing the capability to bind with Neprilysin, which is an enzyme that is responsible for the degradation of Amyloid β peptide in the brain (Takaki et al., 2000, as cited by Li et al., 2018). Moreover, the OB and DL values are above the set criteria, indicating their potential as a drug for AD. Diosmetin and (+)-Catechin not only passed the OB and DL criteria but also exhibited a higher binding affinity with the protein target. However, the two compounds failed to meet the standard for BBB.

A similar observation emerged in Serum Albumin (ALB). Between the two docking compounds of Serum Albumin (ALB), specifically the ethyl oleate and flavoxanthin, only the former docking compound exhibited potential as an anti-AD phytochemical. Ethyl oleate may contribute to the excretion of amyloid-beta from the brain to the bloodstream, thereby mitigating the progression of AD (Li et al., 2018). While flavoxanthin demonstrated higher OB, DL, and binding affinity values, it is unable to pass the blood-brain barrier.

Table 2. Ranking of Anti-AD Potential Based on ADME Profile and Binding Affinity

Putative Protein Target	Docking Compound	BBB Values ≥ -0.3	Binding Affinity	OB Values ≥ 30%	DL Values ≥ 0.18
Neprilysin (NEP)	Genkwanin	-0.24	-7.7	37.13%	0.24
Serum Albumin (ALB)	Ethyl oleate	1.10	-5.7	32.40%	0.19
Thyroid hormone (TTR)	Stigmasterol	1.00	-6.8	43.80%	0.76
	Beta-sitosterol	1.11	-6.8	40.30%	0.85
	Ethyl oleate	1.10	-6.7	32.40%	0.19
	Mandenol	1.14	-4.6	42.00%	0.19

Four out of five docking compounds of thyroid hormone (TTR) exhibited at least a 1.00 BBB value. These include stigmasterol, beta-sitosterol, ethyl oleate, and mandenol, arranged from highest to lowest anti-AD potential. Since four compounds passed the BBB criterion, the rank was decided on binding affinity, which put stigmasterol and beta-sitosterol at the top. However, since their binding affinities are equal, their OB values were taken into account. As stigmasterol had a higher OB value, it was deemed to have the highest anti-AD potential. Since ethyl oleate had a higher binding affinity than mandenol, it was ranked third, after beta-sitosterol. Mandenol was ranked fourth, with the lowest anti-AD potential, and flavoxanthin was not considered due to its inability to pass the blood-brain barrier. Four phytochemicals had anti-AD potential, as their protein target is the thyroid hormone. Evidence suggests that thyroid hormone is capable of up-regulating the expression of neuroserpins in neurons. Neuroserpin is a major inhibitor of tissue plasminogen activator (tPA). This inhibition results in the reduction of plasmin in the brain, an enzyme that degrades amyloid-beta and its plaques (Subhadra, Schaller, & Seeds, 2013). The four phytochemicals with potential anti-AD effects could be experimentally validated to

determine whether they can regulate the thyroid hormone.

The last putative target protein with two docking compounds is matrix metalloproteinase (MMP3), the target protein that is used as a diagnostic biomarker for AD and inhibits the fibrillation of amyloid-beta. Both Ginkgolide B and Ginkgolide J had low BBB values, at -1.56 and -1.15, respectively. While they have high OB, DL, and binding affinity values, their inability to move across the blood-brain brain renders them with little to no anti-AD potential.

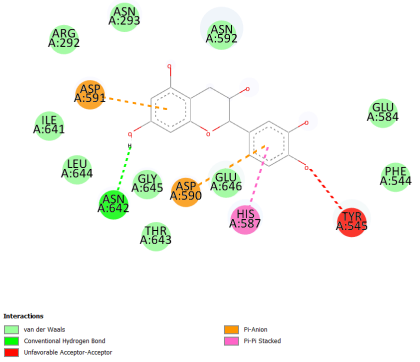

Figure 1. Neprilysin - Catechin Docking Simulation

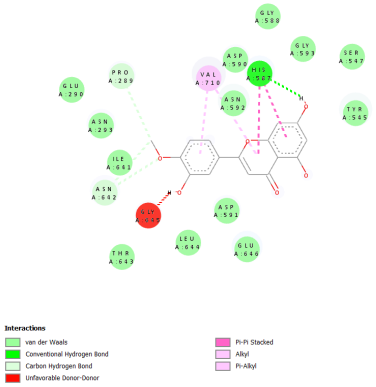

Figure 2. Neprilysin - Diosmetin Simulation

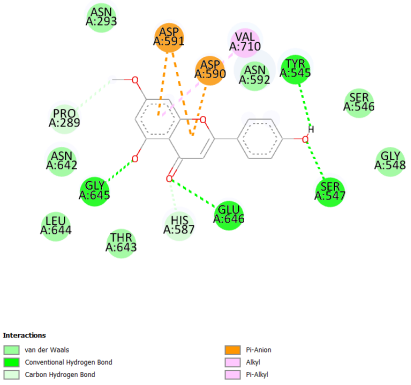

Figure 3. Neprilysin - Genkwanin Simulation

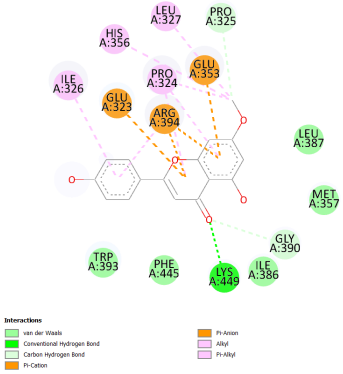

Figure 4. Estrogen Receptor - Genkwanin Simulation

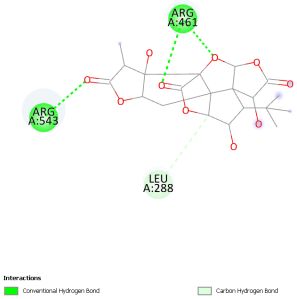

Figure 5. Prothrombin - Ginkgolide J Simulation

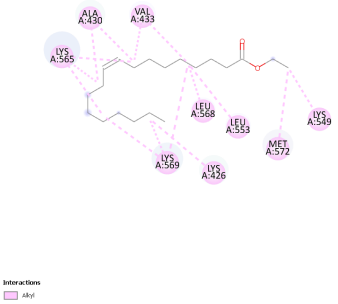

Figure 6. Serum Albumin - Ethyl Oleate Simulation

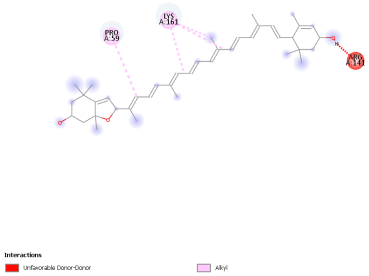

Figure 7. Serum Albumin - Flavoxanthin Simulation

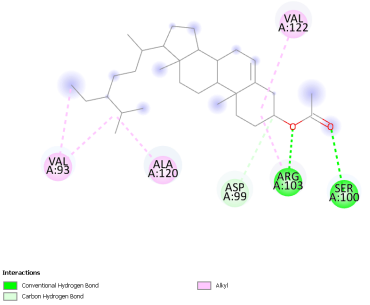

Figure 8. Thyroid Hormone - Beta-sitosterol Simulation

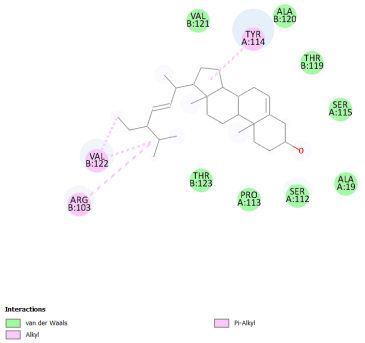

Figure 9. Thyroid Hormone - Stigmasterol Stimulation

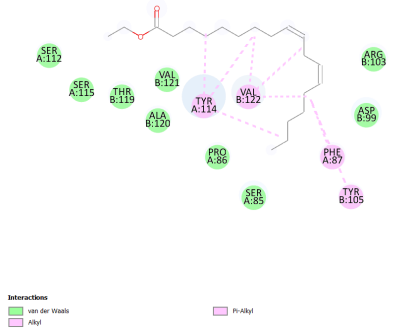

Figure 10. Thyroid Hormone - Mandenol Stimulation

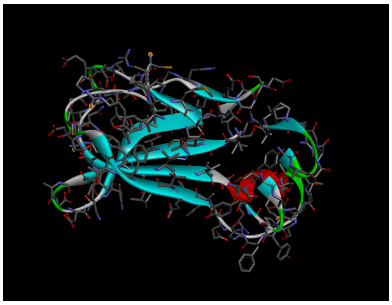

Figure 11. Thyroid Hormone - Ethyl Oleate Stimulation (Ligand is not a single fragment)

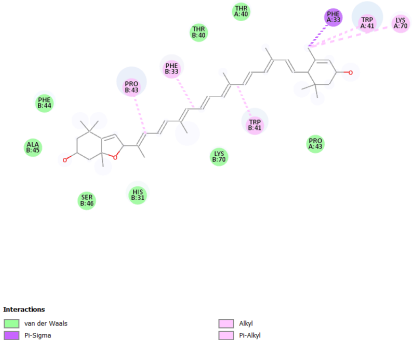

Figure 12. Thyroid Hormone - Flavoxanthin Stimulation

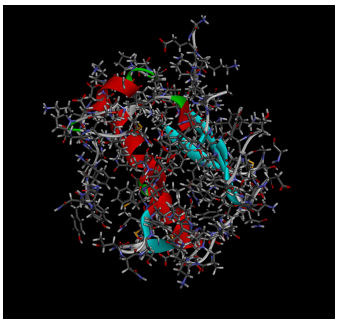

Figure 13. Matrix Metalloproteinase - Ginkgolide B Stimulation (Ligand is not a single fragment)

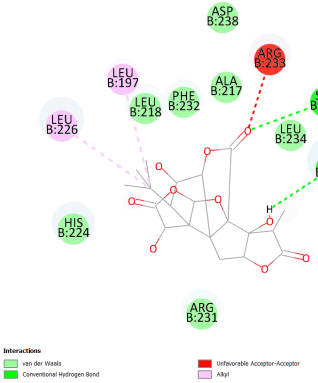

Figure 14. Matrix Metalloproteinase - Ginkgolide J Stimulation

4. CONCLUSIONS

The study emphasized the importance of selecting compounds that meet key pharmacokinetic and pharmacodynamic criteria, particularly BBB permeability, when targeting Alzheimer's Disease (AD). Ginkgo biloba is an example of traditional medicine used to extract the natural compounds that target AD pathology because of their antioxidant, anti-inflammatory, and anti-apoptotic properties (Yang et al., 2015). Because the interaction between these compounds and AD-related proteins has not been studied thoroughly, this study was able to confirm these interactions. The proteins studied were neurolysin, serum albumin, thyroid hormone, and matrix metalloproteinase 3; while the compounds studied were (+)-Catechin, Diosmetin, Genkwanin, Ginkgolide J, Ethyl oleate, Flavoxanthin, Beta-sitosterol, Stigmasterol, Mandenol, and Ginkgolide B. This study delved into the OB values, DL values, BBB values, and binding affinity of these docking compounds. When ranking the docking compounds, compounds with better values for BBB, binding affinity, OB, and DL values were taken into consideration. Given this, the putative protein targets had their respective docking compounds which had anti-AD potential. These are genkwanin for neprilysin, ethyl oleate for serum albumin, and stigmasterol, beta-sitosterol, mandenol, and ethyl oleate for thyroid hormone. In total, five compounds from Ginkgo biloba exhibited anti-AD potential. This study not only confirmed the interactions between Ginkgo biloba compounds and AD-related proteins but also provided a framework for ranking potential drug candidates using these criteria, which is crucial for the development of effective treatments for neurodegenerative diseases like Alzheimer's. It is recommended that the potential of the six phytochemicals be experimentally validated in the laboratory to provide more robust evidence in ameliorating Alzheimer's disease.

5. ACKNOWLEDGMENTS

We would like to acknowledge our friends and family for their unwavering support throughout the conduct of this study.

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