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In Silico Assessment of GSK-3β Inhibition Activity by Secondary Metabolites of *Centella asiatica* in the Development of Alzheimer's Therapy

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Abstract

Alzheimer's disease involves excessive activity of glycogen synthase kinase-3β (GSK-3β), leading to tau hyperphosphorylation and neurofibrillary tangle formation. This study evaluated the potential of secondary metabolites from *Centella asiatica* as GSK-3β inhibitors using in silico molecular docking. The GSK-3β structure (PDB ID: 1Q5K) and ten test compounds were docked using YASARA-structure, with method validation yielding an RMSD of 1.890 Å. Naringin, luteolin, and betulinic acid demonstrated the strongest binding affinities -9.2670, -8.1520, and -7.9730 kcal/mol, surpassing the native ligand. Naringin and luteolin interacted with key ATP-binding residues (Asp133, Tyr134, Val135, Lys85), indicating strong competitive inhibitory potential. These findings suggest that *C. asiatica* metabolites, particularly naringin and luteolin, are promising natural candidates for GSK-3β inhibitor Alzheimer's therapy.

Keywords: *Centella asiatica*; GSK-3β; Molecular Docking; Alzheimer's Disease; Secondary Metabolites

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1 Introduction

Alzheimer's disease is a form of progressive dementia characterized by a decline in cognitive function due to neuronal degeneration in the brain [1]. Data indicate that Alzheimer's is one of the leading causes of death worldwide [2]. However, to date, there is no therapy capable of halting or slowing the progression of Alzheimer's disease. Current treatments are symptomatic, such as acetylcholinesterase inhibitors and NMDA receptor antagonists [3]. One molecular target that plays a crucial role in the pathogenesis of Alzheimer's is the enzyme glycogen synthase kinase-3β (GSK-3β). This enzyme is responsible for the hyperphosphorylation of tau protein and the formation of neurofibrillary tanglesTherefore, the inhibition of GSK-3β has become one of the pathways for the development of Alzheimer's therapy [4].

One potential source for the development of Alzheimer's therapy is the secondary metabolite compounds derived from the plant Centella asiatica, which has been recognized for its antioxidant, anti-inflammatory, memory-enhancing, and neuroprotective activities that can safeguard neurons and improve cognitive function [5], [6], [7]. Administration of C. asiatica extract reduces the expression of GSK-3β and inhibits tau phosphorylation, thereby preventing the formation of neurofibrillary tangles [7].

In silico testing has emerged as an efficient preliminary method for investigating the potential interactions of active compounds (ligand) from *C. asiatica* with GSK-3β through molecular docking analysis. This method facilitates the virtual modeling of interactions between bioactive compounds and target proteins. Through this process, molecular docking can predict the optimal orientation of the ligand when binding to the active site of the protein, as well as calculate critical parameters such as binding free energy and molecular interaction affinity [8]. Consequently, this study is conducted to explore the potential of secondary metabolites from *C. asiatica* as GSK-3β inhibitors using an in silico approach for the development of more effective and nature-based Alzheimer therapies.

2 Materials and Methods

2.1 Materials

The hardware utilized is the ASUS DESKTOP-I1GV5R9, equipped with an Intel® Core™ CPU operating at 3.20GHz (4 CPUs) and 4096MB of RAM. The primary software applications employed are YASARA-Structure version 24.4.10 and Discovery Studio Visualizer (DSV) version 24.1.0.23298, with all software maintained at default settings. The websites and software utilized include the following:

1. Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/) for downloading the receptor protein structures.
2. PubChem (https://pubchem.ncbi.nlm.nih.gov/) for downloading the structures of test compounds and reference compounds.
3. BIOVIA Discovery Studio Visualizer application for ligand and receptor preparation and for visualization of molecular docking results.

The target protein of GSK-3β in PDB format with PDB ID code 1Q5K and its native ligand, n-(4-methoxybenzyl)-n'-(5-nitro-1,3-thiazol-2-yl)urea were obtained from RCSB PDB, and the test compounds naringin, luteolin, glutamic acid, gingerol, ferulic acid, fernesene, ethylpalmitoleat, dihydrocaffeic acid, caryophyllene oxide, and betulinic acid were obtained from PubChem.

2.2 Preparation Ligand and Protein Target

The three-dimensional structure of the GSK-3β enzyme (PDB ID: 1Q5K) was retrieved from the Protein Data Bank and subsequently refined using YASARA-Structure.The native ligand *n*-(4-methoxybenzyl)-*n'*-(5-nitro-1,3-thiazol-2-yl)urea was isolated from the protein structure and stored separately to enable evaluation of the tested metabolites as potential competitive inhibitors. All water molecules and ionic species were removed during this preparation stage. To validate the reliability of the docking workflow, the ligand was subjected to 100 redocking repetitions using a supplementary module within YASARA-

Structure. This preparatory step also involved dissociating the ligand from the receptor and creating the corresponding *1Q5K_receptor.sce* and *1Q5K_ligand.yob* files. Prior to the docking simulations, every compound was further optimized through energy minimization using the steepest-descent algorithm to refine its conformational state.The secondary metabolite compounds found in the leaves of *Centella asiatica* were identified using LC-HRMS, and 35 compounds were found to be present [9]. There are 10 compounds selected for testing in this study, namely naringin, luteolin, glutamic acid, gingerol, ferulic acid, fernesene, ethylpalmitoleate, dihydrocaffeic acid, caryophyllene oxide, and betulinic acid, which were obtained from PubChem. These compounds were then downloaded from the PubChem database.

2.3 Molecular Docking

Yasara-Structure was used for docking in order to view binding energy utilizing the force field scoring method. To accomplish this, a Yasara-Structure platform command called dock_run.mcr must be created. Yasara-Structure automatically creates grid boxes around the native ligand with a radius of 5.0 Å to make docking easier. Receptor residues implicated in the interaction were identified and binding energies were computed using Vina's docking approach integrated into Yasara-Structure. The outcomes are saved in PDB file format (.pdb) following the docking procedure. The Discovery Studio Visualizer program was then used to analyze and visualize the collected data

3 Results and Discussion

Molecular docking is a computational method used to estimate how a ligand fits into a protein's binding site. This technique helps describe how a potential drug interacts with its target protein and how strong that interaction may be. The docking process produces a binding free energy value, which indicates the strength of the ligand and receptor interaction. The crystal structure with PDB ID 1Q5K was chosen as the GSK-3β target model because research shows that blocking GSK-3β can prevent significant pathological processes in Alzheimer's, such as excessive tau protein phosphorylation and the development of neurofibrillary tangles (NFTs) [10]. GSK-3β plays a key role in the pathogenesis of Alzheimer's because its activity can affect Aβ production, tau phosphorylation, neuronal death, and toxic signaling pathways in nerve cells [11]. Therefore, 1Q5K became the target for molecular docking studies of natural compounds as GSK-3β inhibitors to obtain safe and effective new anti-Alzheimer's drug candidates. The stability of a receptor's crystal structure is determined by its resolution. A lower resolution value indicates that the crystal structure in the PDB has a higher structural accuracy, as the positions of the constituent atoms more closely represent their actual state. A structure is considered stable when it has a resolution of less than 2.5 Å. Therefore, the crystal structure 6LU7 used in this study is considered stable and possesses high resolution [12].

Centella asiatica extract has been shown to modulate the GSK-3β pathway, which plays an important role in the pathogenesis of Alzheimer's disease. In that study, administration of C. asiatica extract reduced the expression and activity of GSK-3β, which was followed by decreased phosphorylation of tau protein [7]. Several studies have reported that natural compounds such as flavonoids, polyphenols, and glycosides are able to inhibit GSK-3β thru good binding affinity to the enzyme's active site, and also show neuroprotective potential in Alzheimer's models. Compounds such as quercetin, morin, icariin, genipin, isoorientin, magnolol, and dieckol have been reported as potential GSK-3β inhibitors from natural plants [11]. Additionally, in silico test results for catechin compounds and their derivatives from tea plants also show GSK-3β inhibitory activity[13].

Table 1 Binding energy value and contacting receptor residue from docking result.

No.	Ligand	Binding Energy (kcal/mol)	Contacting Reseptor Residu
1.	Native Ligand	-7.6860	Ile62 Gly65 Ser66 Phe67 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Lys183 Asn186 Leu188 Cys199 ASP200
2.	Naringin	- 9.2670	Ile62 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Tyr140 Arg141 Arg144 Lys183 Gln185 Asn186 Leu188 Cys199 Asp200
3.	Luteolin	- 8.1520	Ile62 Gly63 Asn64 Gly65 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Asn186 Leu188 Cys199 Asp200
4.	Betulinic Acid	-7.9730	Ile62 Gly63 Asn64 Gly65 Val70 Ala83 **Lys85** Thr138 Tyr140 Arg141 Arg144 Lys183 Gln185 Asn186 Leu188 Cys199 Asp200
5.	Ferulic Acid	-6.8910	Ile62 Val70 Ala83 **Lys85** Glu97 Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Gln185 Leu188 Cys199 Asp200 Phe201
6.	Carryophyllene Oxide	-6.293	Ile62 Gly63 Val70 Ala83 **Lys85** Val110 Leu132 Thr138 Arg141 Gln185 Asn186 Leu188 Cys199 Asp200
7.	Dihydro Caffeic Acid	-6.2760	Ile62 Val70 Ala83 **Lys85** Glu97 Val110 Leu132 **Asp133 Tyr134 Val135** Leu188 Cys199 Asp200 Phe201
8.	Gingerol	-6.2210	Ile62 Gly63 Asn64 Gly65 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Gln185 Asn186 Leu188 Cys199 Asp200
9.	Fernesene	-6.1440	Ile62 Gly63 Gly65 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Gln185 Asn186 Leu188 Cys199 Asp200
10.	Ethyl Palmitoleate	-5.7830	Ile62 Gly65 Ser66 Phe67 Gly68 Val70 Ala83 **Lys85** Val110 Leu132 **Asp133 Tyr134 Val135** Thr138 Gln185 Asn186 Leu188 Cys199 Asp200 Gly202
11.	Glutamic Acid	-4.7790	Gly63 Asn64 Gly65 Ser66 Phe67 Gly68 Val69 Val70 **Lys85** Val87 Asp200

Validation of the docking protocol was performed by redocking the native ligand 100 times, which produced a delta RMSD value of 1.890 Å. This result confirms that the docking method used in this study is reliable. Following the validation step, all secondary metabolite compounds from *Centella asiatica* were docked to the target protein. The binding energy obtained from the most stable docking pose of each compound was subsequently used to identify potential lead candidates. Table 1 summarizes the binding free energy values and the interacting amino acid residues for both the native ligand and 10 evaluated metabolites.

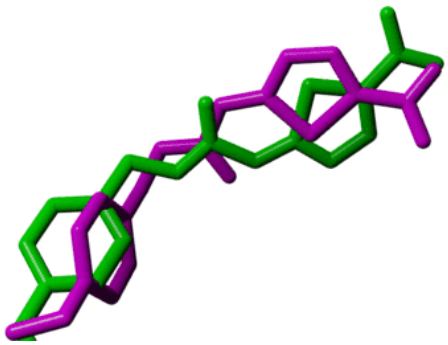

Figure 1 Overlapping Structures from Redocking Results

Based on the results of molecular docking, three secondary metabolite compounds from Centella asiatica, namely naringin, luteolin, and betulinic acid, showed the lowest binding energy values compared to other compounds, with –9.2670; –8.1520; and –7.9730 kcal/mol, respectively. Lower binding energy values indicate a stronger binding affinity to the GSK-3β active site, making these three compounds potentially more effective inhibitors [14]. The binding energy values of the three compounds are lower than that of the native ligand, which has a score of 7.6860 kcal/mol. The compounds naringin and luteolin interact with key residues in the ATP binding pocket of GSK-3β, such as Asp133, Tyr134, Val135, and Lys85. These four amino acid residues are important residues for the binding of competitive inhibitors to the 1Q5K structure. The involvement of these residues indicates that naringin and luteolin are able to bind at the same position as the native ligand, potentially inhibiting GSK-3β activity [11]. While betulinic acid did not show complete interaction with all four residues. This compound only makes contact with Lys85, making it highly unlikely that the ligand occupies the optimal position in the ATP pocket. The Lys85 residue plays a role in forming polar interactions on the ATP-binding side, while Asp133, Tyr134, and Val135 are part of the hinge region, which is the main anchor point for competitive inhibitors. Compounds capable of binding to all four of these residues tend to be more stable at the center of the active site [15].

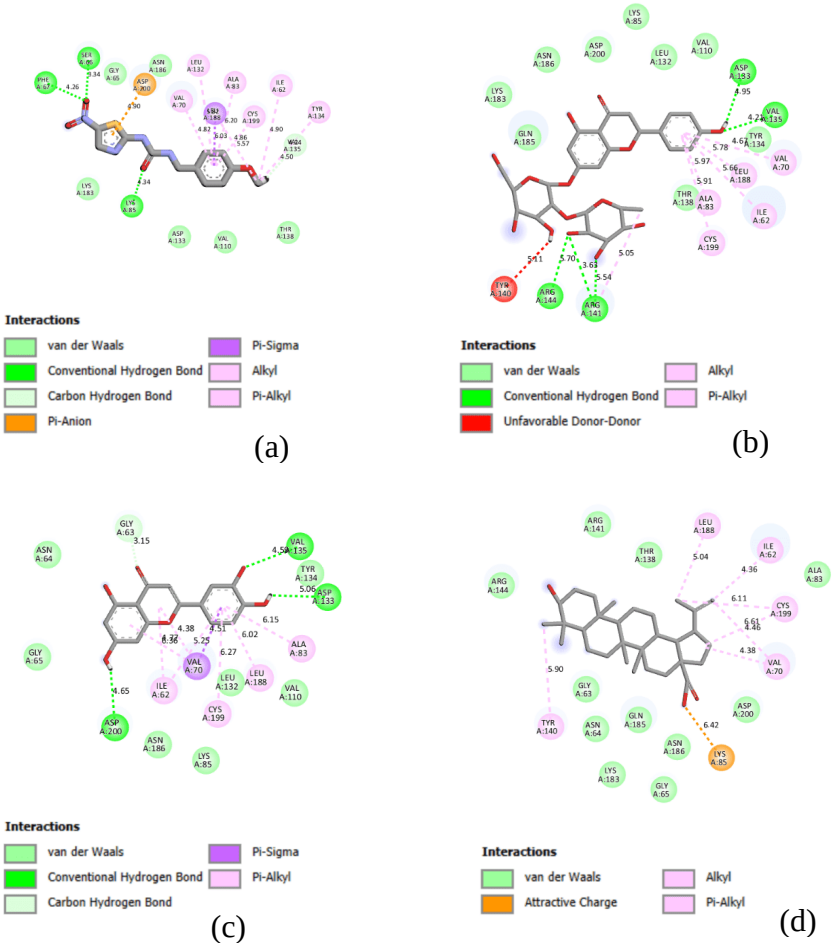

Figure 2 (a) Visualization of the interactions between the native ligand and amino acid residues; (b) Visualization of the interactions between naringin and amino acid residues; (c) Visualization of the interactions between luteolin and amino acid residues; (d) Visualization of the interactions between betulinic acid and amino acid residues

The other seven test compounds did not show lower binding energy values compared to the native ligand. However, this does not indicate that these compounds have no potential activity against GSK-3β. Higher or less negative binding energy values generally indicate weaker affinity, but as long as the ligand can still form some important contacts with residues around the active site, the ligand can still occupy the pocket and exert a mild inhibitory effect [16].

The overall results of this study indicate that secondary metabolites of Centella asiatica have potential as candidate GSK-3β inhibitors for Alzheimer's therapy. These in silico results are also consistent with previous biological data showing that C. asiatica extract is able to reduce GSK-3β expression and inhibit tau phosphorylation in Alzheimer's animal models via the PP2A/GSK-3β pathway [7]. Thus, the high interaction affinity of naringin and luteolin compounds toward GSK-3β in this study provides a strong molecular basis for their neuroprotective activity. Although some other compounds did not show binding energies as low as the initial ligand, the presence of significant interactions at residues around the active site suggests that the secondary metabolites of C. asiatica still retain potential inhibitory activity at weak to moderate levels, making them potential candidates for development as lead compounds [16].

4 Conclusions

The docking results show that the secondary metabolite compounds of Centella asiatica, particularly naringin and luteolin, have high affinity for GSK-3β and are potential

competitive inhibitors based on their free binding energy values and interactions with key residues in the active site. These findings support the potential of C. asiatica as a relevant natural product candidate in the development of Alzheimer's therapy based on GSK-3β inhibition.

5 Declarations

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5.2 Author contributions

Wiwin Azariani contributed to the conception, design, data collection, data analysis, and manuscript drafting as the primary author. Agus Dwi Ananto and Selvira Anandia Intan Maulidya provided supervision, guidance, critical revision, and approval of the final manuscript as academic supervisors.

5.3 Conflict of Interest

The authors affirm that this publication is free from any conflicts of interest. No financial or non-financial interests, personal relationships, or institutional affiliations have affected the preparation, analysis, or interpretation of the findings reported in this study. Any sources of funding, where applicable, have been disclosed transparently, and the research was carried out independently without commercial or organizational influence.

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