

Chemical Profiling and In Silico Study of *Russelia equisetiformis* Major Phytochemicals as Potential Dual-Site Acetylcholinesterase Inhibitors in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory impairment, associated with cholinergic dysfunction and β -amyloid aggregation. Acetylcholinesterase (AChE) plays a dual role in AD progression by hydrolysing acetylcholine (ACh) at the catalytic active site (CAS) and promoting β -amyloid aggregation at the peripheral anionic site (PAS), making dual-site inhibition a promising therapeutic strategy. This study aimed to evaluate the potential of major phytochemicals from the stem of *Russelia equisetiformis* as dual-site AChE inhibitors using an *in silico* approach. Chemical profiling of the ethanolic stem extract was performed using Liquid Chromatography-Mass Spectrophotometry (LC-MS), and compounds with relative concentrations above 2% were selected for further analysis. Molecular docking was conducted using Autodock Vina implemented in PyRx against AChE (PDB ID: 4M0E), while ligand interactions were visualised using BIOVIA Discovery Studio Visualizer. Druglikeness was evaluated using SwissADME based on Lipinski's Rule of Five, and Molecular Initiating Event (MIE) prediction was performed using ProTox 3.0. Docking validation produced an RMSD value of 1.672 Å, indicating acceptable reliability. Among the identified compounds, tilianin exhibited a binding affinity of -8.5 kcal/mol and interacted with key PAS residues (Tyr72, Trp286, and Tyr341) as well as residues extending toward the CAS, comparable to that of donepezil (-7.4 kcal/mol). Tilianin also fulfilled all Lipinski criteria and was predicted to be active toward AChE with a probability score of 0.65. These findings suggest that tilianin is a promising dual-site AChE inhibitor candidate that may simultaneously improve cholinergic neurotransmission and reduce β -amyloid (A β) aggregation. Nevertheless, further *in vitro* and *in vivo* validation studies are required to confirm its therapeutic potential against AD.

Keywords: Alzheimer's Disease; Acetylcholinesterase; Dual-Site; Molecular Docking; *Russelia equisetiformis*.

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia, accounting for 60-70% of cases worldwide, and represents a major age-related public health challenge [1], [2]. More than 55 million people currently live with dementia, with approximately 10 million new cases reported annually, and the prevalence of AD is expected to double every 20 years until 2050 [3].

Clinically, AD is characterized by progressive memory impairment, cognitive decline, mood disturbances, and behavioral abnormalities, which severely impact quality of life [4]. One cause of Alzheimer's disease is a reduction in cholinergic neurons in the brain, leading to decreased acetylcholine (ACh) production. At the same time, acetylcholinesterase (AChE) activity remains elevated, accelerating ACh breakdown and further impairing neurotransmission, thereby contributing to cognitive decline [5], [6].

In addition to cholinergic dysfunction, the accumulation of β -amyloid (A β) peptides plays a crucial role in AD pathogenesis. These peptides aggregate to form neurotoxic oligomers and senile plaques. AChE has a unique structural feature: a deep, narrow catalytic gorge (20 Å) consisting of two important binding regions, namely the catalytic active site (CAS) and the peripheral anionic site (PAS). The PAS region located at the entrance of the gorge

is known to interact with A β peptides and promote their aggregation, thereby accelerating plaque formation. Furthermore, previous studies have shown that ligands that interact with both the CAS and PAS regions can effectively block AChE catalytic activity and inhibit A β aggregation. This dual-site interaction is considered a promising therapeutic strategy to target one of the pathological hallmarks of AD and improve cholinergic neurotransmission [7], [8].

Current Food and Drug Administration-approved treatment for AD, including donepezil, rivastigmine, and galantamine, acts as a cholinesterase inhibitor to alleviate symptoms [9]. However, these synthetic drugs are limited by hepatotoxicity, reduced efficacy over time, and adverse side effects [10]. Therefore, alternative treatments for Alzheimer's disease that are more effective, exhibit minimal side effects, possess low toxicity, and are environmentally friendly, such as those utilizing natural ingredients, are required [11]. A growing body of evidence highlights the potential of phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds as effective AChE inhibitors with additional antioxidant and anti-inflammatory properties [12], [13], [14], [15].

Within this context, *Russelia equisetiformis* Schltdl. & Cham., commonly known as the firecracker plant, has attracted interest due to its rich phytochemical profile. Traditionally used to treat inflammation, fever, diabetes,

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malaria, and leukemia [16], this ornamental shrub contains flavonoids, alkaloids, steroids, tannins, triterpenoids, glycosides, and saponins with reported antioxidant, antimicrobial, hepatoprotective, and antiproliferative activities [17], [18], [19]. These bioactive constituents suggest that *R. equisetiformis* may also possess neuroprotective potential, making it a promising candidate for further exploration as a natural source of AChE inhibitors.

Several studies have reported the AChE inhibitory activity of flavonoids and flavonoid glycosides from various plant sources [20], [21]. However, investigations focusing on phytochemicals from *R. equisetiformis* as dual-site AChE inhibitors remain very limited. To the best of our knowledge, no previous study has comprehensively combined LC-MS-based chemical profiling with molecular docking, druglikeness assessment, and molecular initiating event (MIE) prediction to evaluate major phytochemicals from *R. equisetiformis* against both the PAS and CAS regions of AChE. Therefore, this study addresses an important research gap by identifying potential dual-site inhibitors from *R. equisetiformis* and providing preliminary evidence for their application in AD drug discovery.

Research Methods

Tools and Materials

The tools and materials used in this experiment are *R. equisetiformis* collected from Batu City, East Java, Indonesia, ethanol 96%, filter paper, Buchner funnel, Erlenmeyer side pipe 1000 mL, vacuum pump (Wiggins, Germany), rotary vacuum evaporator (Rotavapor® R-180 Buchi, Switzerland), Liquid Chromatography-Mass Spectrophotometer (8040, Shimadzu, Japan).

Hardware and Software

The hardware and software used were an HP laptop (Intel® Core™ i3-6006U CPU @ 2.00 GHz, 4 GB DDR4 RAM, SSD storage capacities of 128 GB and 512 GB, Intel® HD Graphics 520, Windows 10 64-bit operating system), Autodock Vina and OpenBabel implemented in PyRx Virtual Screening Tool software, BIOVIA Discovery Studio Visualizer, SwissADME, Protein Data Bank (PDB), and PubChem.

Procedure

Determination of *R. equisetiformis*

Fresh samples of *R. equisetiformis* were collected in Batu City, East Java, Indonesia, and taxonomically authenticated by the Generasi Biologi Indonesia Laboratory, Gresik, East Java. Voucher specimen was deposited to provide a permanent herbarium record, ensuring accurate plant identification, traceability of plant materials, and reinforcing the reliability of the research [22].

Extraction of *R. equisetiformis* stem

The stem samples of *R. equisetiformis* were cleaned, dried, and ground into powder. Extraction was performed

using ethanol as the solvent for 24 hours, then repeated 3 times at room temperature. The resulting extract was sequentially filtered and concentrated with a vacuum pump and a rotary vacuum evaporator.

Chemical profiling

Ethanol extract of *R. equisetiformis* was analyzed by Liquid Chromatography-Mass Spectrophotometer (LC-MS) equipped with a Shimadzu Pack FC-ODS capillary column (2×150 mm, $3 \mu\text{m}$ particle size) under isocratic elution with 90% ethanol at 0.5 mL/min, 35°C . Mass spectra (m/z 10-1500) were acquired and metabolites identified by comparison with the NIST database.

In Silico

The dominant compounds ($> 2\%$) from the ethanol extract of *R. equisetiformis* were selected as ligands. The dominant compounds ($> 2\%$ relative concentration) were selected because those present at higher abundance are more likely to contribute significantly to the extract's biological activity and represent the major phytochemical constituents detected by LC-MS [23]. Their 3D structures were retrieved from PubChem (www.pubchem.ncbi.nlm.nih.gov) and subjected to energy minimization using OpenBabel in software PyRx to obtain flexible molecular conformations [24]. The minimized ligands were converted from the structure data format (.sdf) to the Protein Data Bank (.pdb) format. Donepezil, a known AChE inhibitor, was employed as the positive control. The receptor protein used was AChE (PDB ID: 4M0E) [25], [26], obtained from the RCSB database (<https://rcsb.org>) and prepared in BIOVIA Discovery Studio to generate a clean protein structure. Redocking is performed to validate the docking method, with an RMSD $< 2 \text{ \AA}$ [27].

Molecular docking was performed using Autodock Vina implemented in PyRx. The setting of the grid box at x: -17.197857, y: -42.376095, z: 25.582333, with grid dimensions of 13.759472, with an exhaustiveness value of 10 and 100 binding poses generated for each ligand. Visualization of compounds with lower binding affinity than the control drug was performed in BIOVIA Discovery Studio Visualizer to identify binding sites and interaction types.

Drug-likeness prediction was performed to assess the compounds' potential as drug candidates using SwissADME (<http://www.swissadme.ch/>) and Lipinski's Rule of Five. The criteria included molecular weight (MW) ≤ 500 Dalton, hydrogen-bond acceptors (HBA) ≤ 10 , hydrogen-bond donors (HBD) ≤ 5 , and lipophilicity ($\log P$) < 5 [28].

Molecular Initiating Event (MIE) prediction for AChE

The MIE prediction for the AChE enzyme was performed using the Protox 3.0 website (<https://tox.charite.de/protox3/>). This analysis aims to identify potential initial interactions between compounds and AChE as a molecular target [29]. The canonical SMILES of the selected compounds were submitted to the platform, and target prediction analysis was conducted to evaluate the likelihood of interaction with AChE. Predictions are reported as activity probabilities ranging from 0 to 1, where higher

values indicate greater confidence in the predicted interaction. Compounds classified as active were considered to possess potential biological activity toward AChE and were subjected to further interpretation alongside molecular docking and druglikeness results.

Results and Discussion

Determination of *R. equisetiformis*

The *R. equisetiformis* plant in this study was authenticated by the Indonesian Biological Generation Laboratory as belonging to the Plantaginaceae, with the voucher specimen stored in the Herbarium under code GEN 1147.

Extraction of *R. equisetiformis* stem

The stems of *R. equisetiformis* were separated and air-dried for three days. After drying, the material was ground into powder, yielding 162 g of dried powder. Then, the dried powder was extracted by maceration with ethanol for 24 hours. This process was repeated three times at room temperature. During maceration, the solvent permeates cell walls and softens cellular structures. This process facilitates the dissolution of soluble substances and the extraction of target compounds. Ethanol was chosen as the solvent for its non-toxic and neutral nature. It is also an effective solvent. Its solubility supports the dissolution of many compounds, including alkaloid bases, volatile oils, glycosides, curcumin, coumarin, anthracinone, flavonoids, steroids, and chlorophyll [30]. The resulting extracts were filtered. They were then concentrated using a rotary vacuum evaporator to efficiently remove solvents while preserving bioactive compounds. Evaporation under vacuum separates the solvent from the solute without the high heat that can destroy the solute [31]. This process yielded a deep-green crude extract.

Chemical profiling

LC-MS analysis of the ethanolic extract of *R. equisetiformis* stem identified total 116 compounds (Figure 1) with several flavonoid glycosides as the major constituents, each with relative concentrations above 2% (Table 1). The most abundant compounds are kaempferol 3-(6"-caffeoylglucoside), genkwanin 4'-alpha-L-arabinopyranosyl-(1→6)-galactoside, kaempferol 7-rhamnoside-4'-glucoside, quercetin 3,7,4'-triglucoside, scolymoside and tilianin. These flavonoid derivatives identified in the ethanolic extract of *R. equisetiformis* have been reported to exhibit inhibitory activity against AChE [21].

Table 1. Major compounds identified by LC-MS

Compound	Retention Time (RT)	Concentration (%)
Tilianin	22.168	203.620
Genkwanin 4'-alpha-L-arabinopyranosyl-(1→6)-galactoside	33.087	225.384

Compound	Retention Time (RT)	Concentration (%)
Kaempferol-7-rhamnoside-4'-glucoside	34.003	208.556
Scolymoside	34.019	204.574
Kaempferol 3-(6"-caffeoylglucoside)	35.508	243.209
Quercetin-3,7,4'-triglucoside	49.892	208.493

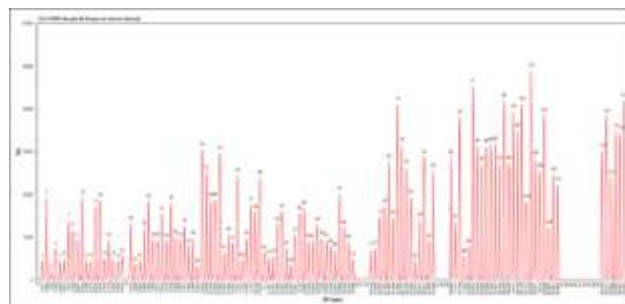


Figure 1. Chromatogram of *R. equisetiformis* stem ethanolic extract

In Silico

Molecular docking simulations were performed on major compounds identified through LC-MS analysis in the ethanol extract of *R. equisetiformis*. Major compounds present at high concentrations in the extract contribute more substantially to pharmacological activity than minor compounds [32]. Molecular docking is performed to assess the compound's inhibitor activity for the AChE enzyme (PDB ID: 4M0E) represented as binding affinity. Binding affinity represents the stability of the ligand-receptor complex [33]. A control docking procedure was conducted by re-docking the native ligand, dihydrotanshinone I, onto the receptor protein 4M0E. The resulting conformation overlapped with the original structure prior to molecular docking, and the root mean square deviation (RMSD) was 1.672 Å (Figure 2). This result indicates that the established docking parameters effectively reproduced the native conformation.

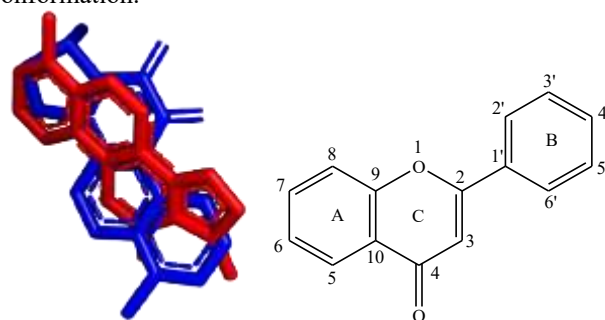


Figure 2. Superimposition of the re-docked native ligand

Docking results show that tilianin has the lowest binding affinity, followed by kaempferol-3-(6"-caffeoylglucoside), genkwanin 4'-alpha-L-arabinopyranosyl-(1→6)-galactoside, and scolymoside, indicating the stronger affinity for the AChE active site, outperforming the standard drug donepezil (Table 2). A molecule with a lower binding affinity value than the control is more likely to be an AChE inhibitor. A lower binding

affinity indicates a more stable ligand-receptor complex and greater potential as an inhibitor. These results suggest that those four compounds may possess promising activity as AChE inhibitors.

Table 2. The binding affinity of major compounds

Compound	Binding Affinity (kcal/mol)
Donepezil (control)	-7.4
Dihydratanshinone I (native ligand)	-11.2
Tilianin	-8.5
Genkwanin 4'-alpa-L-arabinopyranosyl-(1→6)-galactoside	-8.0
Kaempferol-7-rhamnoside-4'-glucoside	-5.1
Scolymoside	-7.7
Kaempferol-3-(6"-caffeoylglucoside)	-8.4
Quercetin-3,7,4'-triglucoside	-3.5

Ligand interactions with the AChE enzyme involve two primary regions, the catalytic active site (CAS) and the peripheral anionic site (PAS). The presence of both sites is critical, as the CAS hydrolyzes ACh, resulting in reduced cholinergic neurotransmission. According to the cholinergic hypothesis, cholinergic function is reduced by approximately 55% in Alzheimer's disease (AD) patients compared to healthy individuals. Cholinergic neurotransmission is essential for memory consolidation and learning, making cholinergic modulation a relevant therapeutic strategy for AD. Additionally, the PAS contributes to the formation of stable complexes with β -amyloid (A β) peptides, thereby accelerating oligomerization and amyloid plaque aggregation [34], [35]. Interactions between AChE and A β have been reported to increase A β aggregation by up to 3-fold compared to A β alone [36]. Notably, inhibitors that target the CAS exclusively, such as Endorphium, do not inhibit A β aggregation, whereas PAS inhibitors, such as Propidium, have been shown to reduce this aggregation [37], [38].

The four selected flavonoid glycosides exhibited interactions with several key residues located within PAS of AChE, particularly Tyr72, Tyr124, Trp286, and Tyr341 (Figure 3). These residues are known to contribute not only to ligand recognition but also to AChE-mediated A β aggregation, making them important targets for dual-site inhibitors. Tilianin formed hydrogen bonds with Arg296, Phe295, and Tyr124 together with a π - π T-shaped interaction involving Tyr72. In addition, van der Waals

interactions with Trp286 and Tyr341 further stabilized the ligand within the binding gorge. This interaction suggests that tilianin can occupy the PAS region while extending toward residues associated with the catalytic gorge.

Kaempferol-3-(6"-caffeoylglucoside) showed hydrogen-bond interactions with Sert293 and Arg296 and established π - π T-shaped interactions with Tyr124 and Tyr341. Similar to tilianin, the ligand also interacted with Trp286 through non-covalent interactions, indicating a favorable orientation within the PAS region.

Genkwanin 4'-alpa-L-arabinopyranosyl-(1→6)-galactoside showed an aromatic interaction forming a π - π stacked interaction with Trp286 and a π - π T-shaped interaction with Tyr124, in addition to multiple hydrogen-bonds with Thr75, Phe295, Tyr337, Tyr341, and Ser293. The interaction with Trp286 is particularly important because this residue is a major component of the PAS and is associated with A β aggregation.

Scolymoside exhibited the highest number of hydrogen-bond interaction, involving Tyr337, Tyr124, Asp74, Ser293, Gln291, Glu292, Thr75, and Tyr72, together with a π - π stacked interaction with Tyr341. The abundance of hydroxyl groups and sugar moieties in scolymoside likely contributes to its extensive hydrogen-bonding network. However, this increased structural complexity also results in a larger molecular size and less favorable druglikeness properties compared to other flavonoid glycosides.

The interaction profile observed for tilianin is consistent with previous studies reporting the AChE inhibitory potential of flavonoids. Al-Hilo (2025) demonstrated that several natural flavonoids, including luteolin and quercetin, exhibited strong binding affinities toward AChE through interactions with both CAS and PAS residues. In particular, flavonoids possessing multiple hydroxyl groups and a planar aromatic scaffold were capable of forming hydrogen bonds and π - π stacking interactions with key residues such as Trp86, Tyr337, and Trp 286, which are important for stabilizing ligand binding within the active gorge [39]. Similar interaction patterns were identified for tilianin in the present study, in which the flavonoid glycoside interacted with Tyr72, Trp286, and Tyr341 in the PAS region and extended toward residues associated with the catalytic site. Wu et al. (2021) reported that naringenin carbamate derivatives exhibited potent inhibitory activity against both AChE and butylcholinesterase (BuChE). Molecular docking analysis demonstrated that these compounds could interact with both the CAS and PAS of the cholinesterase binding pocket, supporting a dual-site inhibition mechanism [40]. These findings suggest that tilianin shares structural characteristics that favor dual-site binding and support its potential as an AChE inhibitor (Figure 3).

Table 3. The chemical structures of selected flavonoid glycosides

Compound	3	5	6	7	4'	5'
Tilianin			-OH	O-Glucoside	O-CH ₃	
Kaempferol-3-(6"-caffeoylglucoside)	O-Glucoside-Caffeoyl	-OH		-OH	-OH	
Genkwanin 4'-alpa-L-arabinopyranosyl-(1→6)-galactoside		-OH		-OCH ₃	O-Galactose-Arabinose	
Scolymoside		-OH		O-Rutinoside	-OH	-OH

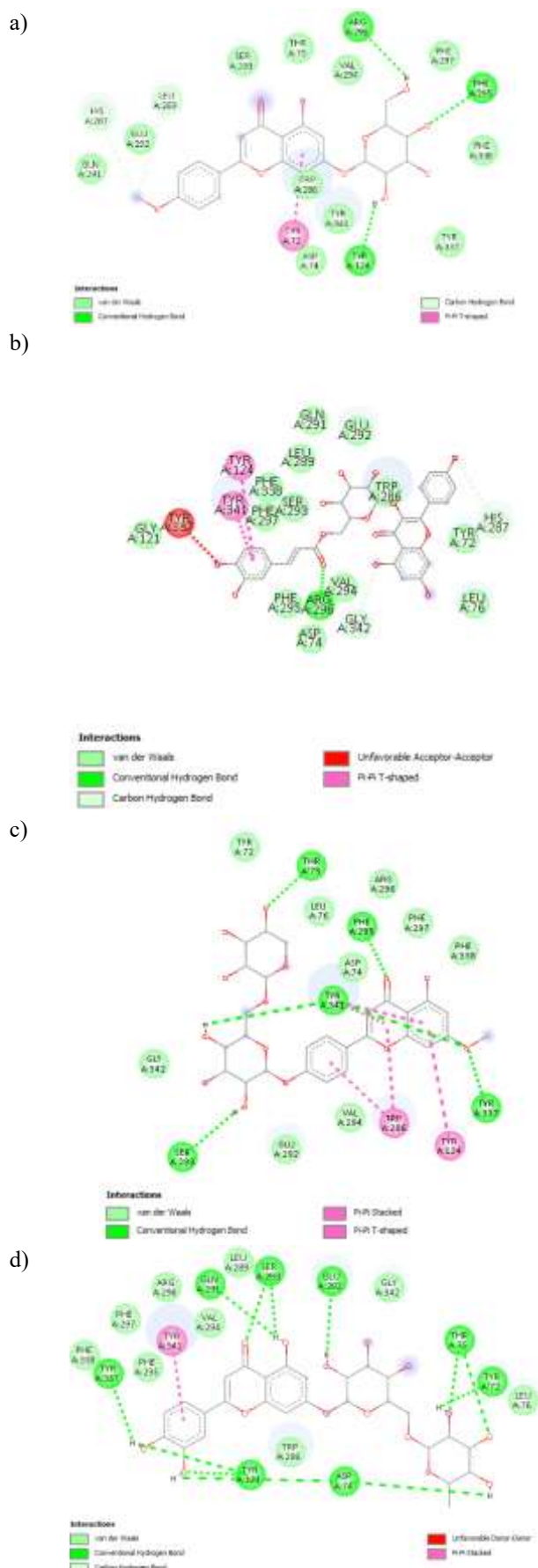


Figure 3. The visualization of the ligand-receptor interactions of (a) tilianin-AChE (b) kaempferol-3-(6''-caffeoylglucoside)-AChE (c) genkwanin 4'-alpha-L-arabinopyranosyl-(1->6)-galactoside-AChE (d) scolymoside-AChE

The differences in binding affinity observed among the four flavonoid glycosides appear to be closely related to their structural characteristics (Table 3). Tilianin exhibited the highest binding affinity, likely due to its structure, which consists of a flavonoid with a single glucose moiety, enabling favorable interactions with key PAS residues. Kaempferol-3-(6''-caffeoylglucoside) exhibited comparable affinity owing to the additional caffeoyl group that enhances aromatic and hydrogen-bond interactions. In contrast, genkwanin 4'-alpha-L-arabinopyranosyl-(1->6)-galactoside and scolymoside contain disaccharide moieties that not only increase hydrogen-bond capacity but also increase molecular size, flexibility, and steric hindrance, potentially reducing binding efficiency. These findings suggest that moderate glycosylation, as observed in tilianin, provides a more favorable balance between receptor interaction and binding affinity than highly glycosylated flavonoids. These findings are consistent with Zhou and Hua (2020), who reported that increased glycosylation of kaempferol derivatives reduced their binding affinity to AChE. The authors suggested that larger steric hindrance associated with glycosylated compounds can impede optimal binding within the enzyme active site [21]. Similarly, in the present study, the monoglycosylated flavonoid tilianin exhibited the highest binding affinity among the diglycosylated flavonoid compounds, suggesting that moderate glycosylation may provide a more favorable balance between receptor interaction and steric effects.

Since the degree of glycosylation was found to influence binding affinity and steric hindrance, further evaluation of the physicochemical properties of the selected compounds is necessary. Therefore, Lipinski Rule of Five analysis was conducted to assess their potential suitability as oral drug candidates. In evaluating druglikeness based on Lipinski's Rule of Five (RO5), an ideal oral drug candidate should have a molecular weight (MW) ≤ 500 Daltons, hydrogen-bond acceptors (HBA) ≤ 10 , hydrogen-bond donors (HBD) ≤ 5 , and lipophilicity (log P) < 5 [28]. A molecule is considered a drug according to the RO5 if it meets three or more of the four RO5 criteria [41].

The results of the RO5 analysis (Table 4) indicate that the compound tilianin has potential as a drug candidate and meets all RO5 criteria. The compounds kaempferol 3-(6''-caffeoylglucoside), genkwanin 4'-alpha-L-arabinopyranosyl-(1->6)-galactoside, and scolymoside each have three violations, so they cannot be categorized as drug compounds. If a drug candidate does not meet more than one of the five Lipinski rules, it does not meet drug-likeness criteria and cannot be considered a candidate for an oral dosage form [42].

The druglikeness profile of tilianin, compared with other flavonoid glycosides, may be attributed to its number of glycoside groups: tilianin has only one glucoside group (monoglycoside), resulting in a lower molecular weight and fewer hydrogen-bond donors and acceptors. Unlike the other glycosylated flavonoids that have two glycoside groups (diglycoside), resulting in a higher molecular weight higher number of hydrogen bond donors and acceptors. The structure of tilianin helps maintain a favorable molecular size and permeability while preserving sufficient hydrogen bonding capacity for receptor interaction. This structure contributes to its compliance with RO5 and supports its potential oral bioavailability.

Table 4. RO5 analysis

Compound	HBA	HBD	MW	Log P
Tilianin	10	5	446.40	-1.39
Genkwanin 4'- α -L-arabinopyranosyl-(1 $\rightarrow$ 6)galactoside	14	7	578.52	-2.96
Scolymoside	15	9	594.52	-3.43
Kaempferol-3-(6''-caffeoyl)glucoside)	14	8	610.52	-1.51

Based on the results of molecular docking and RO5 analysis, the major compound in the ethanol extract of *R. equisetiformis*, tilianin, was identified as having the highest potential as an AChE inhibitor. Tilianin is a flavonoid compound bound to a glycoside, also known as acacetin-7-O-glucoside. Its structure consists of acacetin covalently bonded to glucose at the hydroxyl group in the seventh position through a glycosidic bond [43], [44]. This glucose attachment affects the water solubility, bioavailability, and stability of tilianin due to the presence of the hydroxyl group, compared to its aglycone form, acacetin. Tilianin may help prevent cognitive decline by reducing excitotoxicity and neurotoxic protein aggregation, such as A β [45].

Molecular Initiating Event (MIE) prediction for AChE

The Molecular Initiating Event (MIE) prediction by ProTox 3.0 classified tilianin as active toward AChE with a probability score of 0.65. In the ProTox prediction framework, higher probability values indicate greater confidence in the predicted interaction between a compound and a biological target. The active classification obtained for tilianin supports the molecular docking results, which demonstrated favorable binding affinity and interactions with key residues located at the PAS and toward the CAS region of AChE. The convergence of docking, druglikeness, and MIE prediction results strengthens the hypothesis that tilianin may act as a potential dual-site AChE inhibitor. Nevertheless, computational target prediction remains probabilistic and should be confirmed through experimental enzymatic inhibition assay (*in vitro*) and through *in vivo* assay.

Conclusion

The present study demonstrated that several major phytochemicals identified in the ethanolic stem extract of *Russelia equisetiformis* exhibit potential acetylcholinesterase-inhibitory activity. Among the evaluated compounds, tilianin exhibited the most favorable profile, showing the strongest binding affinity, interactions with key residues associated with the peripheral anionic site and catalytic active site, compliance with the Lipinski Rule of Five, and positive Molecular Initiating Event prediction. These findings suggest that tilianin may act as a dual-site acetylcholinesterase inhibitor, simultaneously enhancing cholinergic neurotransmission and reducing AChE-mediated β -amyloid aggregation, two important therapeutic targets in Alzheimer's disease. The results also highlight the potential of *R. equisetiformis* as a promising natural source of bioactive compounds for developing an anti-Alzheimer agent. However, this study was limited to computational

approaches, and the predicted biological activities require further validation through *in vitro* enzyme inhibition assay and *in vivo* assay to confirm their efficacy, safety, and pharmacokinetic properties.

Author's Contribution

D.A.H.Insyira: responsible for conducting the research, collecting data, and preparing manuscript drafts. Tukiran: contributed to data validation and provided guidance and direction in manuscript preparation.

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