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Original Research Article

Analysis of Active Compounds and *In Silico* Study of *Vigna unguiculata* as an Anti-Alzheimer Disease Agent

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ABSTRACT

β-secretase and butyrylcholinesterase have a role in the pathogenesis of Alzheimer's Disease (AD). AD is a disease characterized by a progressive decline in cognitive, memory function and other important mental functions. This disease often occurs in older people. *Vigna unguiculata* is a Leguminaceae plant known to contain secondary metabolites (flavonoids, phenols, alkaloids, and terpenoids). Previous researchers have proven that *V. unguiculata* has the potential to prevent AD. However, the active compounds (s) that possess anti-Alzheimer's Disease effects are unknown. The research aims to analyze the content of the active ingredients in ethanolic seeds extract of *V. unguiculata* and predict the affinity of the compounds for *β*-secretase (2OHM) and butyrylcholinesterase (4XII) enzymes using the *in silico* method. Active compounds of *V. unguiculata* seeds ethanolic extract were identified using Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS). Affinity predictions of active compounds in inhibiting the enzymes were carried out computationally using Autodoc Vina. Indicators of the affinity of the active compounds for the two enzymes are shown by the binding free energy (ΔG) value and the structural similarity between the ligands compared to natural ligands. Visualisation of docking was done using Biovia Drug Discovery Studio. Quercetin, vitexin, epicatechin, and N-acetyl-DL-tryptophan were some active compounds that showed a lower ΔG and structural similarity between 40-75% compared to the control. The active compounds in *V. unguiculata* seeds extract are predicted to have potential in AD prevention by inhibiting *β* secretase and butyrylcholinesterase implicated in AD.

***Keywords*:** *Vigna unguiculata*, Alzheimer's Disease, *β*-Secretase, Butyrylcholinesterase.

Introduction

Alzheimer's disease is a progressive neurodegenerative disease and is the type of dementia most commonly suffered by older people. This disease is often found in people over 65 years old. It is estimated that the number of AD sufferers will reach 131 million in the world by 2050. The number of AD patients has increased almost 3 times now compared to 2019.[1] The pathomechanism of AD is believed to be caused by intracellular accumulation of amyloid beta peptide (A*β*) and tau protein, increased *β* secretase activity, damages from free radicals, and neurotransmitter disorders (increased glutamate production, decreased acetylcholine activity).[2,3] Symptoms of AD include a progressive decrease of cognitive abilities, a decreased ability for short-term and long-term memory, disorientation in space, time, and people recognition, as well as psychological disorders.[4] The accumulation of A*β* peptide is the primary mechanism for AD occurrence. The *β* secretase cuts amyloid precursor protein (APP) at its ectodomain, producing a soluble APP ectodomain and a C-terminal fragment 99 (C99). Then, the C99 is cut by *γ* secretase, forming the non-soluble A*β* peptide and the amyloid precursor protein intracellular domain (AICD).

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The *α* secretase activity cuts APP, producing APPsa and a C terminal fragment 89 (C89). C89 is then processed by *γ* secretase to produce P3 peptide and AICD. Thus, *β* activities oppose *α* secretase to induce the accumulation of A*β* peptide in the brain.[5]

Free radicals are also said to influence the occurrence of AD.[2] In AD, many free radicals are derived and non-derived from oxygen in neuronal cells in the cortex, hippocampus, and limbic system. An imbalance between free radicals and endogenous antioxidants causes oxidative stress in neuron cells. Free radicals are known to damage membrane lipids, reduce glutamine synthase and creatinine kinase activity, damage DNA, and induce neuron cell death.[2,6] Free radicals Cu^{2+}, Fe^{2+}, and Zn^{2+} have a high affinity for the metal domain of A*β* peptide, so an increase in these metals causes the formation of hydroxyl free radicals. This condition will trigger an inflammatory process and worsen the damage to the nervous system.[7]

Acetylcholine deficiency is also associated with the occurrence of AD. Acetylcholine is an excitatory neurotransmitter produced by neuron cells. In the synaptic gap, acetylcholine will be inactivated by the cholinesterase. High activity of cholinesterase will cause a decrease in acetylcholine activity in the synaptic gap, thereby inhibiting neuronal excitation. There are two types of cholinesterase, namely acetylcholinesterase and butyrylcholinesterase. These two enzymes are homologous. The high activity of butyrylcholinesterase in AD patients has led to the hypothesis that reducing the activity of this enzyme is one method that can be used to inhibit the progression of AD.[8,9]

The public use of herbs to treat various diseases has recently increased. In Indonesia, the communal use of medicinal plants as a treatment for diseases in 2010 was 4.6% and increased to 24.6% in 2018.[2,10] The reason for herbal consumption is due to dissatisfaction with the results of conventional therapy, being accustomed to consuming herbs to prevent or treat disease, considering that herbs are more natural, cheaper, and cause fewer side effects.[11] One plant that has been widely

studied is *V. unguiculata* . *Vigna unguiculata* is a Leguminaceae plant. This plant contains many active compounds beneficial for health, such as proteins, antioxidants, unsaturated fatty acids, and polyphenols. This plant is known to have the effect of reducing the risk of cardiovascular disease and cancer.[12,13] Water extract of *V.unguiculata* significantly reduces malonaldehyde levels and acetylcholinesterase (AChE) activity compared to the control mice with cognitive deficit. These results confirm that *V. unguiculata* has the potential to be an antioxidant and increase acetylcholine activity in the brain.[14]

In silico is a computational research method widely used to predict the mechanism of active compounds causing pharmacological effects. This method has the advantage of being straightforward and fast, reducing costs, and shortening the time spent searching for new drugs from start to market. However, this method also has weaknesses, so it is still necessary to provide *in vivo* verification of the computational prediction results.[15,16] Among *in-silico* methods, molecular docking is one method that is widely used. The molecular docking method predicts the affinity of a small molecule, ion, or atom (ligand) for a natural organic molecule (protein, enzyme, DNA, RNA). Assessment of the ligand and protein affinity using the AutoDock docking tool is based on the total energy calculation value (ΔG) and structural similarity to the control used. The smaller the value (ΔG), the more the structural similarity between the ligand and protein targets compared to the control, and the higher the ligand binding affinity to the target protein.[16]

The study aims to analyse the content of active compounds and predict the affinity of the compounds from *V. unguiculata* seeds ethanolic extract for *β*-secretase (2OHM) and butyrylcholinesterase (4XII) enzymes as treatment options for AD using *in silico* method.

Materials and Methods

Research Design

The research was conducted at the Material Medika Center, Batu City, Saint and Life Sciences Laboratory, Brawijaya University, Malang, Research Laboratory, UNISMA, Malang, from May 2023 to January 2024. It is a laboratory and computational-based study.

Materials

These include a rotary evaporator (Heidolph, Germany), digital scales, a Water shaker (Memmert, Germany), and Oven Binder (Type ED 115, Germany). The phytochemicals of *V.unguiculata* ethanolic seeds were identified using High-performance liquid chromatography (HPLC) (Thermo Scientific Q Exactive, Germany). All chemicals, ethanol, acetonitrile, NaOH, and HCl (Merck, USA) used in this study were analytical grade.

The data processing software was Discoverer with mzCloud MS/MS Library. The docking process used an 11th Gen Intel Core i5-1135G7 2.40GHz processor specifications, RAM 16 GB, and Microsoft Windows 11 Home Single Language version 22H2 64-bit operating system laptop. Autodock Vina version 1.5.7 (https://github.com/ccsb-scripps) was used for the docking study. The docking process was visualised using Discovery Studio Visualizer v21.1.0.20298.

Plant collection and identification

The plant sample (*V.unguiculata* seeds) was collected in May 2023 at Materia Medika Center, Batu City (-7.867707, 112.519271) East Java, Indonesia, identified and a voucher specimen number 000.93/1532/102.20/2024 was assigned.

Preparation of V. unguiculata Ethanolic Extract

The dried seeds sample was extracted by maceration using a modified method of Permatasari *et al*. (2020).[17] *V. unguiculata* seeds powder was placed into an Erlemeyer bottle and dissolved in 70% ethanol in a ratio of 1:5 (w/v). The Erlenmeyer bottle was covered with aluminium foil and shaken for 4 hours using a water bath shaker. The maceration process was carried out for 24 hours at room temperature. The extract was filtered using a Whatmann No. 1 paper, and the filtrate was collected in an Erlenmeyer bottle. This maceration process was repeated 3 times. The filtrate was collected and evaporated at 60°C until the volume reached 10% of the initial volume. The resulting extract was oven-dried at 60°C until a concentrated extract was obtained.[17]

Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) Analysis of Crude Plant Extract

Extract samples were diluted using a polar solvent. The sample was diluted to a final volume of 1500 µL. The sample was vortexed at 2000 rpm for approximately 2 min and spun at 6000 rpm for approximately 2 minutes. The supernatant was then filtered using a 0.22 um filter syringe and placed in a vial. The sample in the vial was inserted into the autosampler and then injected into the LC-HRMS according to the desired injection method. Gradient elution uses a linear gradient system consisting of Solution A containing water and 0.1% Formic Acid and Solution B containing Acetonitrile + 0.1% Formic acid. The column uses Hypersil Gold aQ 50 x 1 mm x 1.9 u particle size. Experimental specifications include instrument flow: 40 mL/min, column oven at 30°C, Sample injection volume: 2 µL, analysis running time: 30 minutes, with full MS at 70,000 FWHM Resolution, Dependent data MS2 at 17,500 FWHM, set at a positive and negative ion modes with Heated Electrospray Ionization (H-ESI). Data were processed using Compound Discoverer with mzCloud MS/MS Library software.[18]

Molecular Docking of Active Compounds From V.unguiculata Ethanol Extract

The ligands used in the docking process were active compounds from *V. unguiculata* seeds ethanolic extract previously identified using LC-HRMS and have 3D structures. The 3D structures of the active compounds were downloaded from PubChem (http://pubchem.ncbi.nlm.nih.gov). *β* secretase (2OHM) and butyrylcholinesterase (4XII) were downloaded from the Protein Data Bank site (http://rcsb.org). The target protein was prepared by removing water molecules and native ligands and then saved in pdbqt format. The native ligand separated from the protein was imported into the Autodock tool to add charges and hydrogen bonds and then saved in pdbqt format. The same process was carried out for the bioactive ligands from *V. unguiculata* seeds extract. The grid box settings were carried out before docking. The grid box was adjusted to the site of the target protein bound to the native ligand. The size of the grid box for the bond between the native ligand and *β* secretase in the x-axis was 25,000Å, y-axis 25.000Å, z axis 25.000Å, x centre 62.277Å, y centre 23.649Å and z centre -40.489Å. The size of the grid box for the bond between the native ligand and butyrylcholinesterase in the x-axis was 25.000Å, y-axis 25.000Å, z-axis 25.000Å, x centre 18.880Å, y-centre 23.649Å, and z centre -40.489Å. Validation was carried out to ensure the native ligand could be used as a control. The validation process was done by redocking the native ligand against the target protein using the Pymol application (Figure 1).[19] The frequency of repetition of the docking process was 8 times. The native ligand is valid as a control if the Root Mean Square Deviation (RMSD) value between the target protein and the native ligand is less than 2Å.[16,20] The docking process was carried out between the active compounds of *V.unguiculuta* extract, *β* secretase and butyrylcholinesterase using Autodock Vina. The docking results of the target protein with the ligands were obtained in pdb format, and the docking results were visualised using the Drug Discovery Studio application.[16,21]

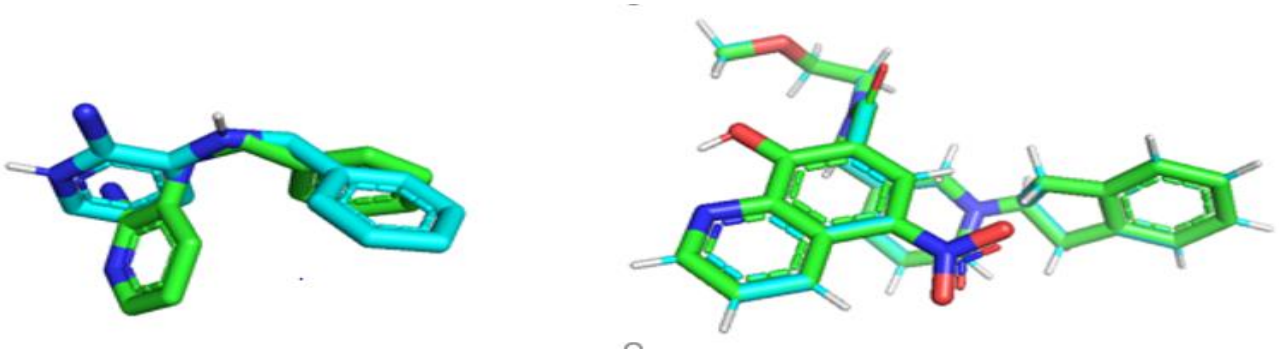
Data Analysis

Descriptive analytical data analysis was based on the value of the free binding energy and structure similarity between ligand and protein compared to control, which can be assessed from the magnitude/percentage of binding to the active amino acid residues of the target protein site compared to the control.[16,19,20,21]

Results and Discussion

The phytochemical compounds of *V.unguiculata* identified from LC-HRMS are shown in (Figure 2). The results show 50 identified active compounds based on a mzCloud Best Match of more than 90% (Table 1). These ingredients consist of protein, amino acids, fatty acids,

organic compounds, phenolic compounds, and flavonoids. The same results were obtained in research conducted by Razgonova (2022).[23] Of the 50 active compounds, it was found that 10 compounds had the most significant area, namely choline (vitamin/mineral), trigonelline (alkaloid), D-Tryptophan (amino acid), L Phenylalanine (amino acid), Trans-3-indoleacrylic acid, NP 009322, Betaine (amino acid), Valine (amino acid), and L-Norleucine (amino acid).



(A) (B)

Figure 1: (A). Validation of the native ligand N-3-Benzylpyridine-2,3-Diamine with the *β*-secretase enzyme, with an RMSD value of 1.66Å. **(B).** Validation of the native ligand with the butyrylcholinesterase with an RMSD value of 0.2Å.

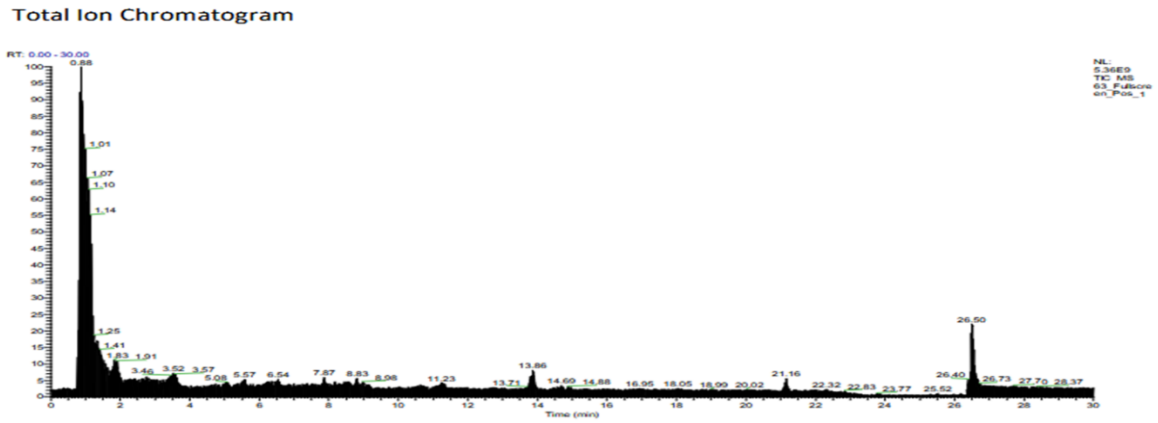


Figure 2: Chromatogram results of active compounds of *V.unguiculata* seed ethanolic extract using the LC-HRMS Method

Table 1: Results of phytoconstituents identified by the LC-HRMS analysis of *Vigna unguiculata* Ethanolic Extract

No	Name	Classification	Formula	Molecular Weight	RT (min)	Area (max)	mzCloud Best Match
1	Choline	essential nutrient	$C_5H_{13}NO$	103. 09	0.967	36,467,414,604.92	97.2
2	Trigonelline	Alkaloid	$C_7H_7NO_2$	137.04	0.904	9,184,050,674.02	99.4
3	D-(+)-Tryptophan	amino acid	$C_{11}H_{12}N_2O_2$	204.08	3.553	1,908,218,010.93	98.7
4	L-Phenylalanine	amino acid	$C_9H_{11}NO_2$	165.07	1.866	1,618,218,433.61	99.1
5	trans-3-Indoleacrylic acid	alpha,beta-unsaturated monocarboxylic acid	$C_{11}H_9NO_2$	187.06	3.532	1,505,356,933.97	96.6
6	NP-002322	monohydroxy fatty acid	$C_{18}H_{32}O_4$	312.22	13.862	1,359,696,032.54	94.7
7	Betaine	amino acid	$C_5H_{11}NO_2$	117.07	0.898	1,014,267,597.79	97.4
8	L-Norleucine	amino acid	$C_6H_{13}NO_2$	131.09	1.405	840,866,379.73	99.6
9	L-Phenylalanine		$C_9H_{11}NO_2$	165.07	1.184	793,685,743.15	98.6
10	3,4-Dimethoxycinnamic acid	polyphenol compound	$C_{11}H_{12}O_4$	208.07	8.817	567,939,963.65	98.2
11	Maltol	fruty sweet	$C_6H_6O_3$	126.03	1.24	558,951,486.32	97.8
12	L-Pyroglutamic acid	amino acid	$C_5H_7NO_3$	129.04	1.185	441,849,494.49	94.8
13	L-Glutamic acid	amino acid	$C_5H_9NO_4$	147.05	1.153	434,675,144.91	96.4
14	9S,13R-12-Oxophytodienoic acid	polyunsaturated fatty acid	$C_{18}H_{28}O_3$	292.20	10.609	394,947,108.33	95.1
15	2,3,4,9-Tetrahydro-1H-β-carboline-3-	alkaloid	$C_{12}H_{12}N_2O_2$	216.08	5.074	343,226,411.99	98.4

No	Name	Classification	Formula	Molecular Weight	RT (min)	Area (max)	mzCloud Best Match
16	carboxylic acid 4-Coumaric acid	phenol compound	$C_9H_8O_3$	164.04	6.501	322,200,232.78	98.3
17	D-(+)-Proline	amino acid	$C_5H_9NO_2$	133.07	0.922	291,240,671.64	99.4
18	Adenosine	endogenous purine nucleoside	$C_{10}H_{13}N_5O_4$	267.09	1.127	275,160,067.14	99.8
19	Nicotinic acid	vitamin B3	$C_6H_5NO_2$	123.03	0.958	200,008,122.25	97.8
20	L-Histidine	amino acid	$C_6H_9N_3O_2$	155.06	0.85	197,796,282.28	98.5
21	Stearamide	fatty acid	$C_{18}H_{37}NO$	283.28	23.794	161,907,817.46	97.1
22	N1-imino(2-methyl-1,3-thiazol-4-dimethyl-4 (trifluoromethoxy)benzanamide	heterocycle containing sulphur and nitrogen atoms	$C_{13}H_{10}F_3N_3O_2S$	329.05	0.902	158,874,129.06	93.1
23	Ferulic acid	phenolic compound	$C_{10}H_{10}O_4$	194.05	7.002	155,003,944.08	98.6
24	L-Tyrosine	amino acid	$C_9H_{11}NO_3$	181.07	1.169	136,304,057.89	94.9
25	Bis(4-ethylbenzylidene)sorbitol	sugar alcohol	$C_{24}H_{30}O_6$	414.20	14.624	136,179,818.43	99.2
26	Vitexin	Flavone	$C21H_{20}O_{10}$	432.10	6.918	126,653,163.52	93.2
27	Indole	aromatic, heterocyclic organic compound	C_8H_7N	117.05	3.53	108,561,871.99	94.5
28	Erucamide	fatty amide	$C_{22}H_{43}NO$	337.33	25.494	107,941,653.49	95.6
29	6-Methylquinoline		$C_{10}H_9N$	143.07	5.077	107,517,084.10	98.2
30	4-Aminophenol	organic compound	C_6H_7NO	109.05	0.984	106,185,112.35	92.2
31	Bis(2-ethylhexyl) phthalate	organic compound	$C_{24}H_{38}O_4$	390.27	23.142	85,568,144.22	99.7
32	Epicatechin		$C_{15}H_{14}O_6$	290.07	2.398	78,958,004.55	99.2
33	Hexadecanamide	Fatty amide	$C_{16}H_{33}NO$	255.25	21.951	54,756,594.48	99.1
34	Dibenzylamine	aromatic amine organic compound	$C_{14}H_{15}N$	197.11	7.394	53,513,793.11	98.8
35	δ-Valerolactam	chemical compound classified as lactam	C_5H_9NO	99.06	1.854	43,410,694.51	94.5
36	Quercetin-3β-D-glucoside	flavonoid	$C_{21}H_{20}O_{12}$	464.09	7.15	41,297,095.63	98.5
37	1-Stearoylglycerol	Long-chain fatty alcohol	$C_{21}H_{42}O_4$	358.30	22.829	40,850,485.88	97.3
38	3',4'-Dimethoxyacetophenone	alkyl-phenylketones compound	$C_{10}H_{12}O_3$	180.07	8.518	39,755,678.32	91.5
39	3-Methoxy prostaglandin F1α	Protein	$C_{21}H_{38}O_6$	408.24	12.77	36,812,069.22	99
40	3-Amino-2-naphthoic acid	unnatural aromatic amino acid	$C_{11}H_9NO_2$	187.06	5.045	36,690,506.51	91..5
41	Oleamide	fatty acid	$C_{18}H_{35}NO$	281.27	21.596	25,324,851.71	93.4
42	Palmitoyl ethanolamide	fatty acid	$C_{18}H_{37}NO_2$	299.28	20.349	20,801,918.42	99.1
43	Linoleoyl Ethanolamide	fatty acid	$C_{20}H_{37}NO_2$	323.28	18.973	19,140,354.96	97.1
44	Caffeine	phenolic compound	$C_8H_{10}N_4O_2$	194.07	4.702	18,983,652.66	98.3
45	(12Z)-9,10,11-trihydroxyoctadec-12- enoic acid	Long-chain fatty acid	$C_{18}H_{34}O_5$	352.22	11.964	18,799,948.54	98.1
46	α-Eleostearic acid	fatty acid	$C_{18}H_{30}O_2$	278.22	16.968	18,464,007.32	92.6
47	Hexamethylenetetramine	heterocyclic organic compound	$C_6H_{12}N_4$	140.10	26.442	13,909,288.98	95.2
48	Quercetin	flavonoid	$C_{15}H_{10}O_7$	302.04	6.23	11,214,810.46	98.1
49	Daidzein	Isoflavone	$C_{15}H_{10}O_4$	254.05	9.086	9,803,152.92	96.2
50	5-(6-hydroxy-6-methyl octyl)-2,5-dihydrofuran-2-one	butenolide organic compound	$C_{13}H_{22}O_3$	248.13	8.176	8,000,137.83	95.8

Description: Table 1. Lists active compounds that have mzCloud Best Match of more than 90%. This study identified 50 bioactive compounds with a mzCloud Best Match of more than 90%. These active compounds consist of protein, amino acids, fatty acids, organic compounds, phenolic compounds, and flavonoids. Ten active compounds had the most significant area, namely choline (vitamin/mineral), trigonelline (alkaloid), D-Tryptophan (amino acid), L Phenylalanine (amino acid), Trans-3-indoleacrylic acid, NP 009322, Betaine (amino acid), Valine (amino acid), and L-Norleucine (amino acid).

Choline was the most abundant active compound identified in *V.unguiculata* seeds ethanolic extract. Yuan *et al*. found that a low choline diet of less than 2 g/day increased the risk of developing dementia and AD.[24] A diet high in choline given early has been proven to improve spatial memory, reduce hyperexcitability, and restore Neuronal Nuclear (NeuN) antigen expression in an AD mouse model. NeuN antigen is a marker of many neurological disorders in which levels decrease in pathological conditions.[25]

Tryptophan is an amino acid thought to be abundant in *V.unguiculata* seeds' ethanolic extracts. The results of research conducted by Savonije and Weaver proved that tryptophan and its metabolites inhibit Aβ amyloid formation, neurotoxic β amyloid oligomerisation, regulate neuroinflammation, and improve sleep disorders that occur in AD patients. However, quinolic acid (tryptophan metabolites) is toxic to neurons, thereby increasing the progression of AD.[26] Trigonelline is the alkaloid most commonly found in *V. unguiculata* seeds' ethanolic extract. Trigonelline from Fenugreek has been shown to have a neuroprotective effect through an antioxidant mechanism. Trigonelline causes increased expression of the antioxidant gene, increasing Superoxide Dismutase (SOD) and catalase activity and scavenger free radical.[27]

The flavonoid compounds in the *V. unguiculata* seeds ethanolic extract are quercetin, epicatechin, vitexin, ferulic acid, daidzein, and caffeic acid. However, the levels of these compounds were low in the extract. Flavonoids are one of the phenolics that are often found in plants. Flavonoids consist of 2 phenol rings (A and B) connected by a heterocyclic ring (C) (Figure 3). Flavonoid activity is linked to changes in the location of rings B and C, the degree of hydroxylation, oxidation, and saturation of ring C. The hydroxylation process is at positions 7 and 5 of ring A. The oxidation occurs at positions 3', 4', and 5' of ring B. There are 10 types of flavonoids in plants (anthocyanin, proanthocyanins, flavonol, flavone, glycoflavone, biflavonyl, chalcone, aurone, isoflavone, and flavanone).[28,29]

Flavonoids are known to have neuroprotective effects. Quercetin is an active compound that can inhibit Aβ aggregation, and tau protein phosphorylation also prevents hydrolysis of acetylcholine by the cholinesterase enzyme.[30] Vitexin has the potential to be neuroprotective because it has an antioxidant effect, reduces inflammation, and prevents phosphorylation of the Tau protein, which is the basis for AD.[31] Epicathechin can prevent AD by inhibiting β-amyloid accumulation, modulating inflammation and stress-associated genes in transgenic strains of *Caenorhabditis elegans*, which express human $A\beta_{1-42}$ peptides.[32]

Fatty acid compounds identified in *V. unguiculata* seeds ethanol extract include 9S,13R-12-oxophytodienoic acid, stearamide, erucamide, 1-stearoylglycerol, oleamide, hexadecanamide, palmitoyl ethanolamide, linoleoyl ethanolamide and (12Z)-9,10,11-trihydroxyoctadec-12-enoic acid. Several researchers have proven the effect of active compounds in the fatty acid group as neuroprotective. Administration of Omega-3 fatty acids is said to be able to prevent neurodegenerative events, reducing the expression of Tumor Nuclear Factor (TNF) α, Cyclooxygenase – 2 (COX-2), and inducible Nitric Oxide (iNOS) in the hippocampus area in AD experimental animals. Other researchers reported that omega-3 fatty acids inhibit the activity of G protein-coupled receptor 43 (GPR43). GPR43 induces oxidative stress and the synthesis of inflammatory mediators. Omega 3 fatty acids are said to inhibit GPR43 activity, thereby preventing the formation of Hydrogen Peroxide (H_2O_2) suppressing neuronal cell apoptosis.[33,34]

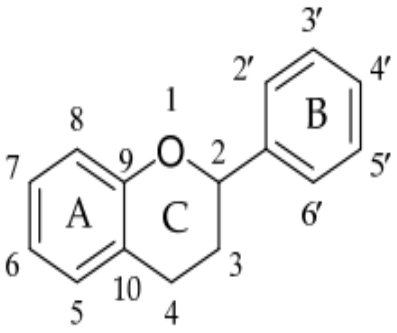


Figure 3: The chemical structure of flavonoid compounds.

The docking results for the affinity of the active compounds of *V.unguiculata* seed ethanolic extract against β secretase and butyrylcholinesterase are presented in Table 2. Visualisation of the docking results between the native ligand as a control and the active compounds from the seeds extract against β secretase and butyrylcholinesterase are shown in Figure 4. The results of redocking between native ligands as a control for β secretase showed a free energy binding of –6.8 kcal/mol and binds to amino acid residues Gln 303, Gln304 Ala335, Arg307, Glu339, Val170, and Tyr14. The active site of the β secretase is located in the amino acid residues Asp31, Try70, Asp227, Gly229, and Ile109 .[35] Other sources reported the active site of β secretase to be Thr133, Gln134, Asn294, Ser386, Leu324, Glu326, Gly325, Thr293, Glu326, Glu371, Arg368, and Lys382.[36] This is possibly due to differences in the protein code used. Docking results between native ligands against the butyrylcholinesterase show a free energy binding of -10.6 kcal/mol and bound to amino acid residues Gly116, Glu197, Gly117, Tyr332, Trp231, and Leu86. Raguel (2022) shows that the active site of butyrylcholinesterase is located in amino acid residues Asp70, Tyr332, Tyr128, Ile442, Trp82, and Glu197.[30] From both studies, it was found that there were similar bonds between the amino acid residues Tyr 332 and Glu197.

The docking results of the active compounds of *V.unguiculata* seed extract showed an affinity with β secretase , showing the four best compounds, including quercetin (-7.6 kcal/mol: 40%), vitexin (-7.3 kcal/ mol: 50%), epicatechin (-7.3 kcal/mol: 60%) and tryptophan (- 7 kcal/mol: 75%) (Table 2). Meanwhile, the four active compounds with the best affinity for butyrylcholinesterase were quercetin (-9.3 kcal/mol: 32%), vitexin (-9 kcal/mol: 32%), epicatechin (-8.9 kcal/mol: 16%), and acetylcholine (-8.9 kcal/mol: 16%) (Table 2). Among *in silico* methods, molecular docking is the most frequently used in the drug discovery process. The basic principle of molecular docking is the ligand and receptor compatibility to form a complex and produce an effect or affinity. The indicators of affinity used in this research were based on the value of the free binding energy and structure similarity between ligand and protein compared to the control (which can be assessed from the magnitude/percentage of binding to the active sites of the amino acid residues of the target protein compared to the control. Free binding energy is released when a compound reacts to produce a complex and stability. The binding to active site amino acid residues shows the percentage of structural similarity between the ligand and the control. The greater the rate of bond similarity with the active site amino acid residues of the target protein, the more similar a ligand is to the control in binding to the target protein.[15,16,22] This study shows that quercetin, vitexin, epicatechin, and tryptophan have lower free energy than the control. All of the bioactive compounds bind to the amino acid residues of the active site of the β secretase (40-70%). Likewise, the docking results of the butyrylcholinesterase show that quercetin, vitexin, epicatechin, and acetylcholine have lower free binding energies and the percentage of bonds with the active site amino acid residues of the butyrylcholinesterase was 16-32%. These two results show that the active compounds have anti-AD effects by inhibiting β secretase and butyrylcholinesterase; however, they are less potent than the control.

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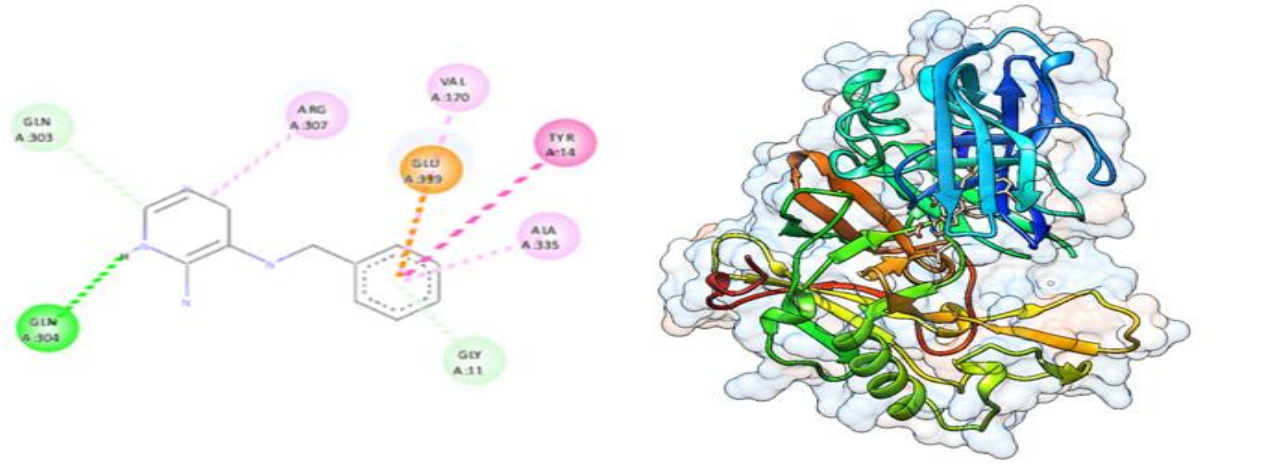
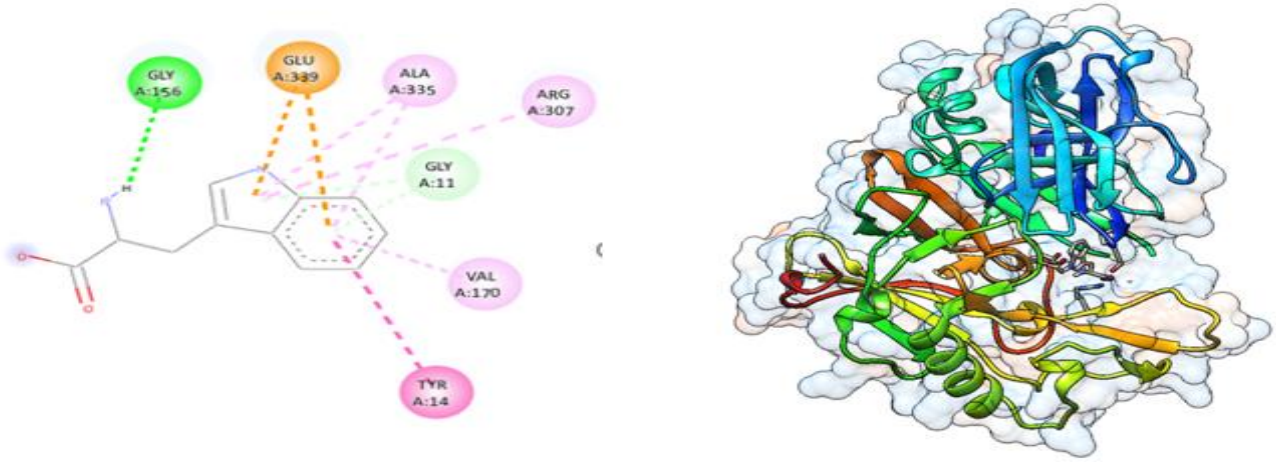
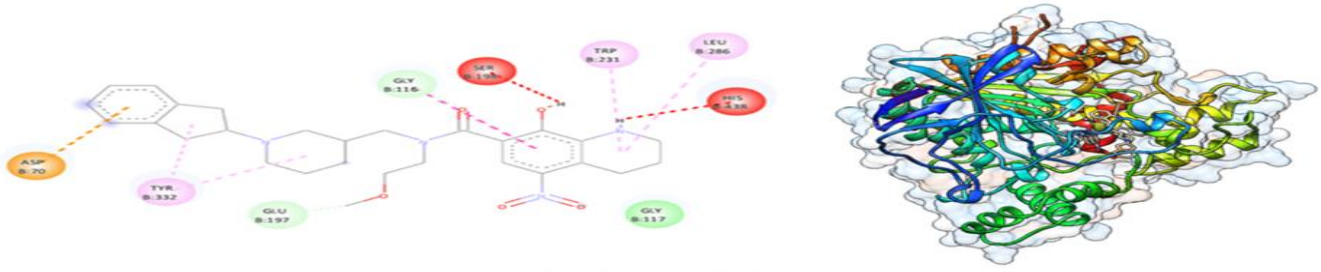


A. Native Ligand- β Secretase



B. Tryptophan – β Secretase

C. Vitexin –β Secretase



D. Native ligand - Butyrylcholinesterase

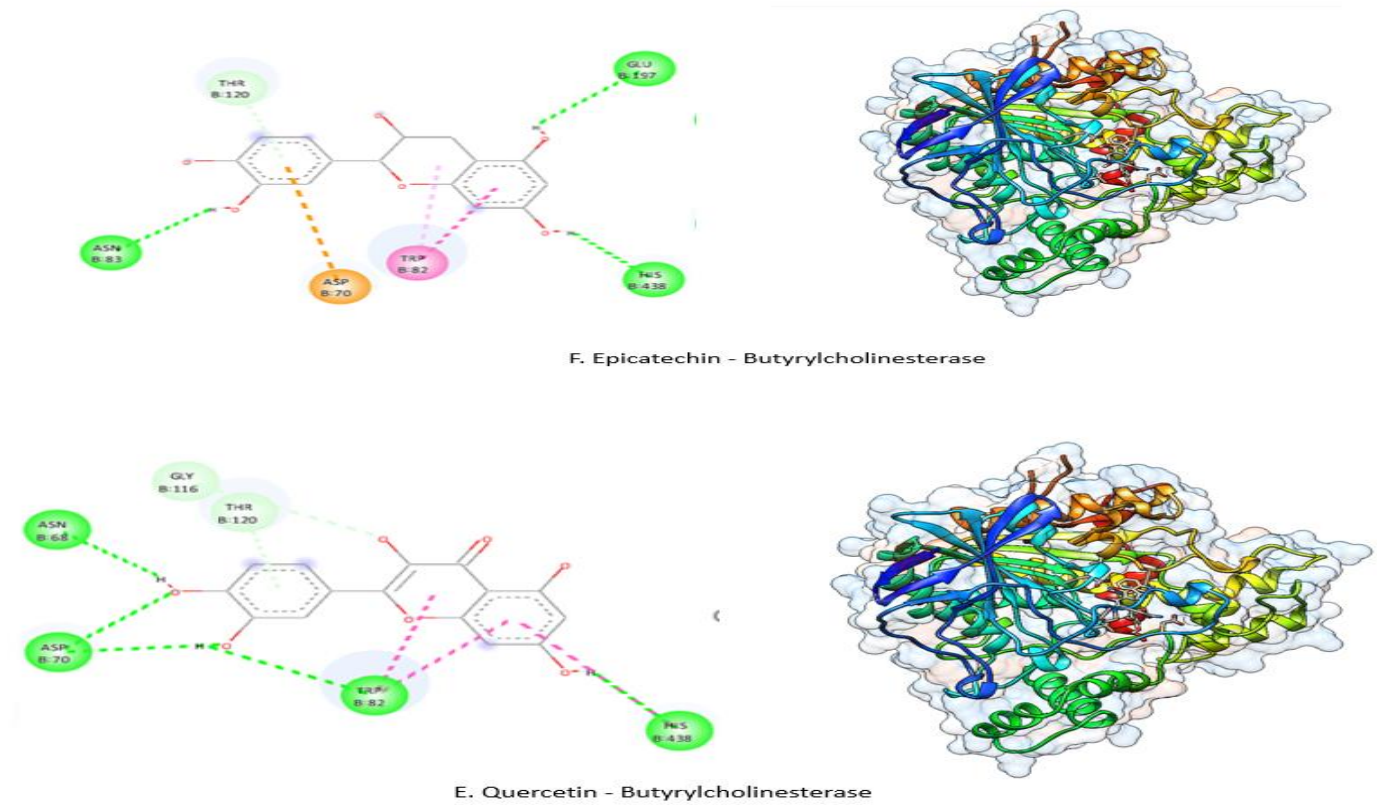


Figure 4: Visualisation of the molecular interaction between the native ligand and the

Table 2: Docking results of active compounds from *V. unguiculata* seed ethanolic extract against the *β*-secretase (2OHM) and butyrylcholinesterase (4XII) as Control

Active Compounds	**CID**	**Free Energy Binding (kcal/mol)**	**Amino acid residues**	**% bonding of amino acid residues compared to native ligan**
BETA SECRETASE (2OHM) benzylpyridine-2,3-diaminel	OHM	-6.8	Carbon Hydrogen bond : GLN303 Convensional hydrogen bond : GLN304 Hydrophobic bond: ARG307, VAL170,GLU339, TYR14, AIA335, GLY11	100

Quercetin	5280343	-7.6	Carbon hydrogen bond :- Convensional hydrogen bond : GLY156, SER10 Hyhidrophobic bond: GLU339, PRO308, ARG307, ALA335, VAL361	40
Vitexin	5280441	-7.3	Carbon hydrogen bond : GLY156 Convensional hydrogen bond :GLN304 Hydrophobic bond: PRO308, VAL361, ALA335, ARG307, GLU339	50
-)-Epicatechin	72276	-7.3	Carbon hydrogen bond : - Conventioanl Hydrogen bond :GLN303, ALA157 Hydrophobic bond :PRO308, VAL381, ARG307, ALA335, GLU339, GLY11	60
N-Acetyl-DL-tryptophan	2002	-7.0	Carbon hydrogen bond: GLY11 Conventional hydrogen bond : GLY156 Hydrophobic bond :ALA335, ARG307,GLU339, VAL170,TYR14	75
BUTYRYLCHOLINESTERASE (4 XII)				
N-{[(3R)-1-(2,3-dihydro-1H-inden-2-yl)piperidin-3-yl]methyl}-8-hydroxy-N-(2-methoxyethyl)-5-nitroquinoline-7-carboxamide	4XII	-10,6	Hydrogen bond: GLY116,GLU197,GLY117 Hydrophobic bond: TYR332,TRP231,LEU286	100%
Quercetin	5280343	-9,6	Hydrogen bond: GLY116,THR120,ASN68,ASP70,TRP82,HIS438 Hydrophobic bond: -	32%
Vitexin	5280441	-9,0	Hydrogen bond: TRP82, GLY117 Hydrophobic bond: LEU286	32%
-)-Epicatechin	72276	-8,9	Hydrogen bond : THR120,ASN83, GLU197, HIS438 Hydrophobic bond: TRP120	16%
6-Acetylcodeine	5486550	-8,7	Hydrogen bond: ASP70, THR120,GLY117, HIS438 Hydrophobic bond: TRP 82	16%

Description: Table 2 lists the docking results of active compounds against target proteins with free binding energy less than the control and -7 kcal/mol. The Docking process uses the Autodock Vina application. Letters in bold indicate identical amino acid residues as the control. The results of redocking between native ligands as a control for protein *β*-secretase (OHM) obtained free energy of – 6.8 kcal/mol and bonds to amino acid residues GLY75, GLY156, ALA335, ARG307, GLU339, VAL170, TYR14. Based on the free energy values and the percentage of bonds to amino acid residues, 4 active compounds of *V. unguiculata* ethanolic extract were obtained to have an affinity for the *β*-secretase enzyme, namely Quercetin, vitexin, epicatechin, and N Acetyl tryptophan. The potency of these 4 compounds is lower than the control. The results of redocking between native ligands as a control for butyrylcholinesterase (4XII) obtained free energy of -10,6 kcal/mol and bonds to amino acid residues GLY116, GLU197, GLY117, TYR332, TRP231, LEU286. Based on the free energy binding values and the percentage of bonds to amino acid residues, 4 active compounds of *V. unguiculata* ethanolic extract were obtained to have an affinity for the *β*-secretase enzyme, namely Quercetin, vitexin, epicatechin, and 6-Acetylcodeine. The potency of these 4 compounds is lower than the control.

Conclusion

The study revealed that quercetin, vitexin, epicatechin, tryptophan, and acetylcholine in the seeds ethanolic extract of *V. unguiculata* have affinities for the *β* secretase and butyrylcholinesterase enzymes with a potency lower than the control. The absence of anti-AD mechanisms exhibited by the most abundant compounds contained in the seeds extract, such as choline and trigonelline in this study, opens up opportunities for further research to explore other mechanisms of these active compounds to prevent AD both *in silico, in vitro* and *in vivo* in AD animal models.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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