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Article

Molecular Simulation for Screening Bioactive Compounds as Potential Candidate for Alzheimer's Disease

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Abstract. Alzheimer's disease is one of the neurodegenerative diseases that afflict the elderly. One of the symptoms is a loss cognitive ability due to neuronal death caused by amyloid plaque accumulation. Alzheimer's disease is one of the most expensive diseases to treat. Drugs for Alzheimer's treatment only treat the symptoms, not the disease itself. Several pathways, including the mitochondrial cascade, can be used to develop drugs for Alzheimer's disease, according to NIH guidelines. Caspase3 is a protein that involved in the mitochondrial cascade, specifically in apoptosis. Alzheimer's therapy may be more effective if caspase3 is targeted. Indonesia is a rich country, particularly in medicinal plants. We used the Structure-Based Drug Design approaches to screen bioactive compounds in Indonesian medicinal plant to find the best compound candidate. In addition, we performed ADMETOX prediction, molecular docking, and molecular dynamic simulation on forty 3D structures of bioactive compounds and donepezil as an FDA approved Alzheimer's drug. We discovered Miraxanthin-V had a higher binding affinity than donepezil using molecular simulation. As a result, we can conclude that Miraxanthin-V has a high potential of neuroprotective by inhibiting apoptosis.

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1. Introduction

Alzheimer's disease is a neurodegenerative disease that causes cognitive decline in people over the age of 65 [1-2]. Alzheimer's disease is caused by the accumulation of beta-amyloid (Aβ) protein and tau protein hyperphosphorylation. Amyloid plaques form in the extracellular spaces of nerve cells. The plaque is formed by the clumping of proteins Aβ 40 and 42 [1][3]. Protein Aβ accumulation is thought to be capable of causing tau-dependent synaptic dysfunction. The tau protein is found within the intracellular space of nerve cells [1][4]. The presence of tau protein stimulates the activity of NMDA receptor. When Aβ protein accumulates in the extracellular space, tau protein accumulates in the nerve cell's intracellular space [5-6]. This causes an influx of calcium ions in the dendrite, resulting in the death of nerve cells [3][7]. One of the proteins involved in the death process is the caspase-3 protein, which acts as the death executor in the apoptotic pathway [8-9].

Caspase-3 expression is elevated in Alzheimer's patients [9-11]. This is because caspase-3 is a death-executor protein involved in the apoptosis process. Caspase3 is involved in both intrinsic and extrinsic apoptotic pathways [3][8]. Furthermore, caspase3 is involved in neuronal plasticity and synaptic loss [11-12]. Caspases 3 inhibition is thought to aid in the process of restoring post-synaptic composition [3][9]. Based on market-available Alzheimer's therapy drugs, the NIH proposes nine research targets for Alzheimer's treatment, one of which is through the mitochondrial cascade [13-14]. The mitochondrial cascade is closely linked to apoptosis and autophagy, both of which require the caspase-3 protein [15-17].

Alzheimer's drugs are currently classified into two types: acetylcholinesterase inhibitors and NMDAR antagonists [12][18]. These two types of medications can only help with the symptoms of Alzheimer's disease; they cannot cure it [20-21]. Furthermore, when these two drugs are taken, they cause a variety of side effects, including nausea, vomiting, and loss of appetite [7][14].

Herbal plants are one of Indonesia's natural resources [22-23]. Locals commonly use herbal plants to increase stamina, maintain immunity, and treat diseases [24-25]. These herbal plants are commonly combined to make jamu, a drink. The drink is made from a variety of medicinal herbal plants [22] [26].

Many researchers have studied the bioactive compounds found in herbal plants over the last few decades [24][26]. Some of these bioactive compounds may have the potential to treat diseases like neurodegenerative diseases [27-29]. With advances in bioinformatics science, it is now possible to identify drug candidates that will save time and money. Drug candidates are discovered in bioinformatics using molecular simulations such as screening, molecular docking, and molecular dynamics [27][30]. The target of Alzheimer's drug discovery from Indonesian herbal plants is hoped to be discovered using a molecular simulation approach.

2. Experimental Section

The experimental was done using the previous pipeline for drug discovery processes. The process includes virtual screening from databases and molecular simulation using molecular docking and molecular dynamics. [26][29-30]. It may be seen on Figure 1.

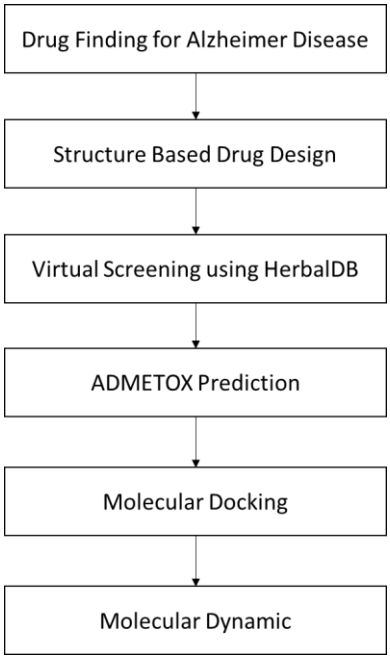

Figure 1. Virtual screening and molecular simulation for drugs finding

2.1. Materials and Methods

This research we used laptop with I7 specification. For protein target screening we retrieved protein from KEGG, while compound we got from HerbalDB. Screening is done using LigandScout. Molecular docking is done using Autodock Tools 4.0, while visualization 2D is done using LigPlot+. Molecular dynamic is done using Gromacs.

2.2.1. Protein Preparation

The caspase3 protein was chosen by searching the UniProt database (https://www.uniprot.org/) with the criteria "Homo sapiens" and choosing structure part. From structure part we found the valid PDB ID for caspase3 and sorted the protein by clicking the RCSB-PDB database (https://www.rcsb.org/). Resolution, year of publication, presence of mutations, and presence of native ligands were the criteria used to select the best protein of caspase3. Proteins that met these requirements were downloaded in .pdb format.

2.2.2. Bioactive Compound Screening and Preparation

The HerbalDB database, which contains 1337 compounds, was used to screen the bioactive compound database. The LigandScout software was used for virtual screening by uploading the PDB structure of caspase3 and the HerbalDB database. Structure-based was done by selecting the yellow box of active site. Data from virtual screening was then collected in Microsoft Excel.

2.2.3. Bioactive Compound's Structure Retrieval from Databases

The PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://www.chemspider.com/), and DrugBank (https://go.drugbank.com/) databases were used to determine the structure of the bioactive compounds. The screened compounds were then searched for in PubChem and ChemSpider for download in .sdf format. Positive control was an Alzheimer's therapy drug that can be downloaded from DrugBank in the .sdf format.

2.2.4 ADME and Toxicity Prediction

The SWISS ADME (http://www.swissadme.ch/index.php) web server and ADMETlab 2.0 (https://admetmesh.scbdd.com/) were used to predict ADME and toxicity. Smiles from bioactive compounds were uploaded into the web server, and information on ADME and toxicity was obtained. Lipinski's 5 rules and the severity of toxicity were the parameters used to find the best candidate bioactive compound.

2.2.5. Molecular Docking Preparation

AutodockTools software was used for molecular docking. Prior to molecular docking, bioactive compound, that have been sort from ADMETOX prediction, were prepared by converting the .sdf format to the .pdb format using MarvinSketch software. Following that, bioactive compound and caspase3 protein were prepared in AutodockTools software to produce the .pdbqt file format.

2.2.6. Molecular Docking Validation

Validation was done by making the grid box from different sizes (40x40x40, 50x50x50, and 60x60x60). Native ligand, which obtained from protein caspase3, was used to do the validation test in each size. We need to find the best box size by finding the smallest RMSD after docking with native ligand.

2.2.7. Molecular Docking Simulation from Bioactive Compounds

The validated grid box was then used to perform molecular docking between the target protein and the screened bioactive compounds. Variables that were considered binding energy and inhibition constant. After molecular docking simulation has been done, complex binding from caspase3 protein and bioactive compound was built to perform the 2D visualization. 2D visualization was conducted using LigPlot+ by uploading the complex binding in .pdb format.

2.2.8. Molecular Dynamic

The GROMACS software was used to simulate molecular dynamics. To perform molecular dynamics, bioactive compounds and caspase3 protein preparation must be completed using vector machine in GROMACS software. Water and ions were added after the preparation stage was finished. Then there was energy minimization, pressure and temperature additions, and molecular dynamics simulations. The calculation of RMSD and RMSF was obtained from molecular dynamics. Microsoft Excel could be used to visualize RMSD and RMSF value.

3. Results and Discussion

3.1 Protein Preparation

The caspase3 protein was chosen from the RCSB-PDB database (https://www.rcsb.org/) based on resolution, year of publication, mutations, and native ligands. The resolution of protein is measured in Armstrong (A) units. The resolution parameter is important because the lower the resolution, the clearer the appearance and depiction of atoms [31-33]. A resolution of 3.00-4.00 A is only capable of describing the rough structure of a protein, while 2-3 A can describe the finer structure of a protein, which is distinguished by the appearance of a rough aromatic ring shape [30][34]. A resolution of 1-2 A can describe a very fine protein structure with the appearance of an aromatic ring shape. As a result, the lower the resolution, the more clearly the protein structure is represented [31][35].

The year of protein was published is important for identifying amino acid residue groups [36-37]. The amino acid residue group is more renewable the more recent the year. This must be taken into account to avoid the loss or omission of an amino acid residue group [38-39]. Because they can bind to the ligands being tested, amino acid residue groups are important in molecular simulation [33][37][39].

Mutations may cause changes in amino acids and change the conformation of a protein [34][40-41]. This conformational change has the potential to alter protein's function and properties [33][42]. A protein with no mutations is required in molecular simulations to represent the protein in its original state [16][41]. Another factor to consider is the presence of native ligands [21][38]. Native ligands are those carried by proteins. In the presence of native ligands, the binding sites on these proteins and the amino acid residues that play a role in these binding sites can be predicted [37][43].

Based on this information, 2xyh was chosen as a candidate protein that reflects the parameters that must be considered (https://www.rcsb.org/structure/2XYH). The caspase3 protein 2xyh was discovered experimentally using the X-Ray method. The 2xyh protein was first published in RCSB-PDB in 2010, with a resolution of 1.89 A. There were no mutations found in either chain of the 2xyh protein. TQ9 is a 2xyh protein native ligand [33]. Figure 2 depicts the structure and information of the 2xyh protein.

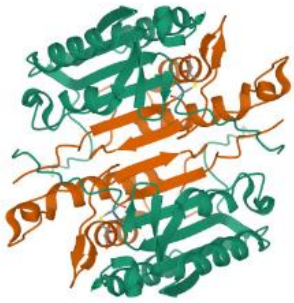

Figure 2. RCSB-PDB structure and information for the 2xyh protein [44]

3.2 Screening of Bioactive Compound

Structure-Based Drug Design (SBDD) is a method for identifying candidate compounds that have the 3D structure of the target protein [32][33]. The search for candidate compounds based on (SBDD) emphasizes the binding site of protein [21][30]. At the binding site, certain amino acid residue groups were discovered to play an active role in the binding of a candidate compound [34][39].

The screening for bioactive compounds was carried out using HerbalDB. HerbalDB is a database that contains a collection of bioactive compounds derived from medicinal plants in Indonesia. HerbalDB is a University of Indonesia pharmacy that houses approximately 1337 bioactive compounds [27][44-46].

When the target protein has a 3D structure, the SBDD method can be used to scan bioactive compounds. Pharmacophores will be identified based on their binding to the protein's binding site, as shown in Figure 3. The active site is typically occupied by a native ligand, which is also a small molecule [39][47]. Table 1 shows the results of scanning bioactive compounds using the SBDD method.

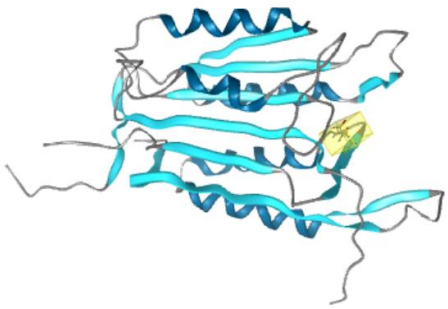

Figure 3. SBDD on 2xyh protein

Table 1. Scanning results of bioactive compounds based on SBDD

Bioactive Compound Name
Batatic acid.mol
Gallic acid.mol
L-(+)-Tartaric acid.mol
Chlorogenic acid.mol
Phaseolic acid.mol
Miraxanthin-V.mol
4-Methylpentyl glucosinolate.mol
3-Methylpentyl glucosinolate.mol
3-Butenylglucosinolate.mol
Suberic acid.mol
Citric acid.mol
9-Methylthiononyl glucosinolate.mol
n-Hexyl glucosinolate.mol
8-Mehtylthio-octyl glucosinolate.mol
Sinalbin.mol
Glucoraphenin.mol
4-Methylsulfinylbutyl glucosinolate.mol
5-Hexenyl glucosinolte.mol
Shikimic acid 3-phosphate.mol
Isoscutellarein 4'-methyl ether 8-(2'',4''-disulfatoglucoronide.mol
Pimeilc acid.mol
Glucoputranjivin.mol

A search of the HerbalDB database yielded 23 potential bioactive compounds. Using molecular docking on the 2xyh protein, the best candidate for bioactive compounds was then identified. The first step is to validate the grid box at 40x40x40, 50x50x50, and 60x60x60 [48][46]. Grid box validation was accomplished by re-docking the native ligand to the binding site of the 2xyh protein. The RMSD (root mean standard deviation) of the re-docking should be less than 2 [38][27]. The dimension with the lowest RMSD number will be used later in molecular docking. This is done to ensure that the bioactive compounds occupy the binding site of the 2xyh protein binding site precisely and produce the desired pharmacological effect [38][46]. Grid box validation on 2xyh protein was performed at dimension 40, as shown in Table 2.

Table 2. Validation Grid Box 2xyh

Grid	Binding Energy	Reference RMSD
40	-4.95	1.14
50	-4.95	1.15
60	-4.94	1.13

To perform molecular docking of bioactive compounds, ligands and 2xyh protein were prepared. Ligand preparation includes torsion selection and regulation, whereas protein preparation includes charge addition, hydrogen atom addition, and the incorporation of non-polar components [47][48]. Table 3 shows the results of molecular docking. The 3 best docking score from binding energy is n-hexyl glucosinolate (-6.62), Miraxanthin-V (-6.58), and Sinalbin (-6.3). While the positive control (donepezil) has docking score -5.37.

Table 3. Docking results of SBDD bioactive compounds

No	Bioactive Compound Name	Binding Energy	RMSD	Inhibition Constant
1	Batatic acid	-4.95	53.776	236.67
2	Gallic acid	-4.81	49.632	299.85
3	L-(+)-Tartaric acid	-4.48	51.002	516.96
4	Chlorogenic acid	-5.93	49.271	45.16
5	Phaseolic acid	-4.71	48.068	351.98
6	Miraxanthin-V	-6.58	51.784	15.11
7	4-Methylpentyl glucosinolate	-5.57	59.224	82.6
8	3-Methylpentyl glucosinolate	-3.48	56.545	2.82
9	3-Butenylglucosinolate	-3.93	55.643	1.32
10	Suberic acid	-3.84	57.236	1.54
11	Citric acid	-4.33	52.77	665.47
12	9-Methylthiononyl glucosinolate	-4.36	49.004	631.38
13	n-Hexyl glucosinolate	-6.62	54.65	13.95
14	8-Methylthio-octyl glucosinolate	-4.4	54.51	596.84
15	Sinalbin	-6.3	51.293	23.94
16	Glucoraphenin	-5.38	55.741	113.29
17	4-Methylsulfinylbutyl glucosinolate	-6.26	55.391	25.98
18	5-Hexenyl glucosinolate	-5.32	57.84	126.22
19	Shikimic acid	-5.15	51.53	166.61
20	Isoscutellarein 4'-methyl ether 8-(2'',4''-disulfatoglucoronide)	-4.04	52.726	1.09
21	Pimelic acid	-4.1	56.949	991.35
22	Glucoputranjivin	-4.47	56.084	562.85
23	Donepezil	-5.37	59.185	115.07

Both the parameter binding energy (Gibbs) and the inhibition constant indicate that the bonds are strong (pKia) [14][50]. The stronger the bond between protein and bioactive compounds, the lower the binding energy, and the lower the inhibition constant, the better bioactive compounds' ability to induce pharmacological effects [51][32]. The inhibition constant is calculated using the equation principle described below:

$$Enzyme + Substrate \leftrightarrow Enzyme - Substrate$$

This equation will result in the formation of complexes between ligands and proteins as a result of the molecular docking interactions that have just occurred. Based on the reaction rate, the following formula can be used to calculate the degree of dissociation [52][32].

$$k = \frac{[Enzyme][Substrate]}{[Enzyme - Substrate]}$$

This equation shows that a high value for the enzyme and substrate complex results in a low degree of dissociation. When the degree of dissociation is low, the substrate complex forms more easily [32][40]. This also occurs during molecular docking between bioactive compounds and 2xyh protein, where the smaller the dissociation constant, the less dissociation. This also shows that the bioactive compound complex with 2xyh protein forms more easily [52][50]. The formation of this complex and binding energy has a significant effect on the action of a drug. The drug can carry out its pharmacological effects effectively if the complex is easily formed and easily dissociated. However, serious side effects will occur if the drug candidate has a strong bond and complex formation that is fast but not easily dissociated [14][32].

Figure 4 show the hydrogen bond in docking result. There were 5 hydrogen bonds in native ligand, 3 hydrogen bonds in control positive, 5 hydrogen bonds in Miraxanthin-V, 3 hydrogen bonds in Sinalbin, and 8 hydrogen bonds in N-hexyl glucosinolate. The hydrogen bond indicates the strength of ligand attachment to protein target. The more the hydrogen bonds identified, the stronger the strengthen it would be.

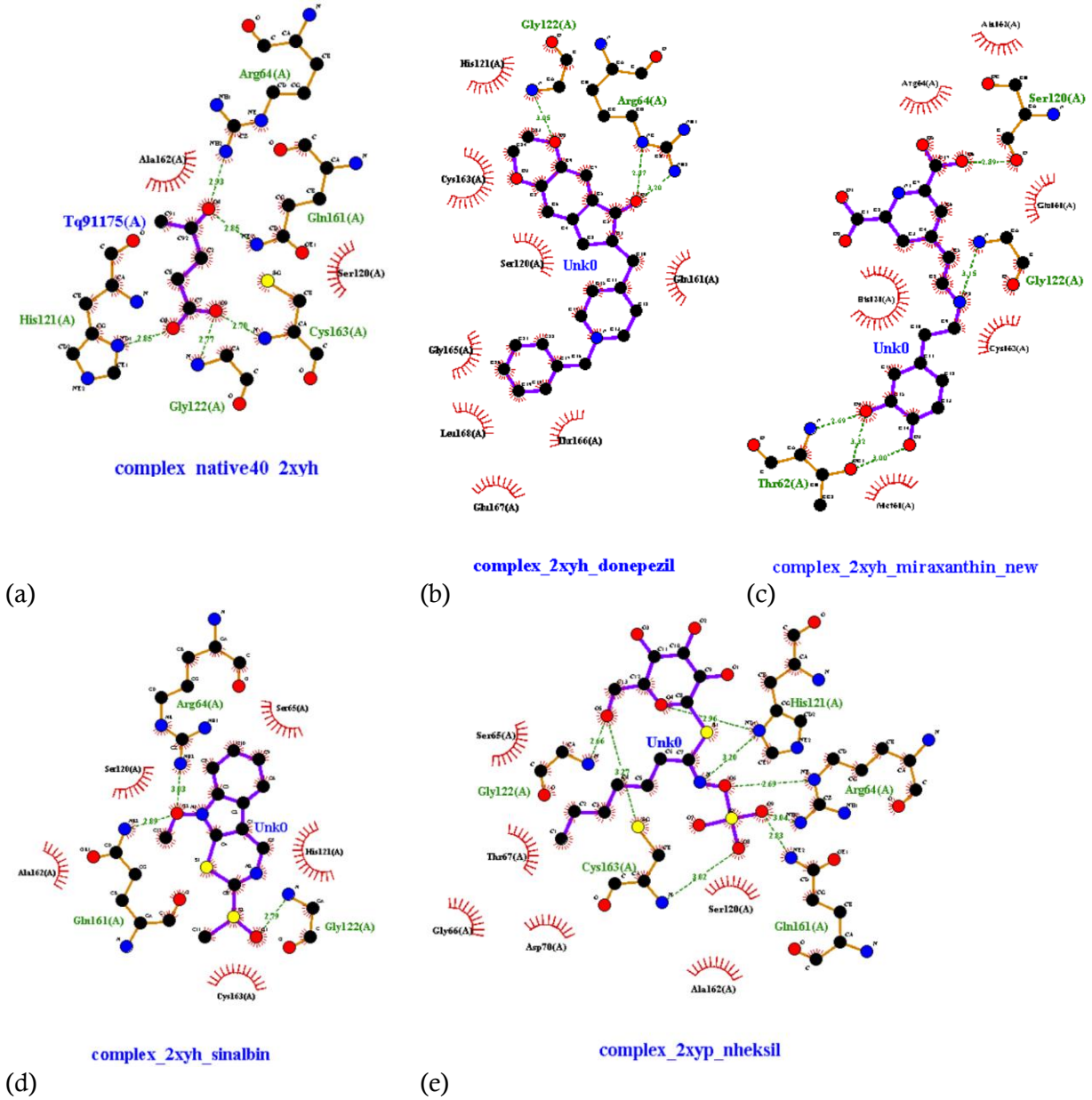

Figure 4. 2D visualization docking results (a) Native Ligand, (b) Positive Control, (c) Miraxanthin-V, (d) Sinalbin, (e) N-hexyl glucosinolate

The molecular docking also yielded amino acid residues, indicating the site of interaction between bioactive compounds and 2xyh protein. One of the parameters that will be used in molecular dynamics simulations is amino acid residue, which will be reflected on the RMSF curve. The amino acid residues in 2xyh protein are shown in Table 4.

Table 4. Amino acid residue 2xyh

Protein	Bioactive Compound	Amino Acid Residue															
		Arg64	Ala162	Gln161	Ser120	His121	Gly122	Cys163	Gly165	Leu168	Thr166	Met161	Thr62	Ser65	Thr67	Gly166	Asp70
2xyh	n-heksil glucosinolate	√	√	√	√	√	√	√	-	-	-	-	-	√	√	√	√
	miraxanthin	√	√	√	√	√	√	√	-	-	-	√	√	-	-	-	-
	sinalbin	√	√	√	√	√	√	√	-	-	-	-	-	√	-	-	-
	donepezil	√	-	√	√	√	√	√	√	√	√	-	-	-	-	-	-
	native	√	√	√	√	√	√	-	-	-	-	-	-	-	-	-	-
Total		5	4	5	5	5	5	4	1	1	1	1	1	2	1	1	1

Prior to entering the molecular dynamics simulation stage, toxicology and ADME predictions on bioactive compounds were performed. The ADMETOX test results are shown in table 5. Table 5 shows that Miraxanthin-V, N-Heksyl glucosinolate, and sinalbin all meet the Lipinski Rule of 5 and are not toxic [32][21]. Toxicity testing is required because we want to conduct the best drug candidate [53][39]. While some molecules are toxic, this can be managed by modifying the structure of the compound using the QSAR method [34][36]. The three compounds were then subjected to 10 ns of molecular dynamics testing to determine binding stability in human-like environmental conditions such as the addition of water, ions, pressure, and temperature. The RMSD parameter indicates the stability of the binding between bioactive compounds and 2xyh protein [54][26]. Figure 5 displays the RMSD of the five compounds.

Table 5. ADMETOX bioactive compounds at 2xyh by Druglikeness

Compound	Druglikeness			
	Molecular Weight g/mol	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Log P
Miraxanthin-V	347.34	6	6	0.03
n-Hexyl glucosinolate	402.46	10	4	-0.14
Sinalbin	424.42	11	5	-0.99
Donepezil	379.49	4	0	4

Table 6. ADMETOX bioactive compounds at 2xyh by Pharmacokinetics

Compound	Pharmakokinetics						
	GI absorption	BBB permeant	Inhibitor CYP				
			CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Miraxanthin-V	Low	No	No	No	No	No	No
n-Hexyl glucosinolate	Low	No	No	No	No	No	No
Sinalbin	Low	No	No	No	No	No	No
Donepezil	High	Yes	No	No	No	Yes	Yes

Table 7. ADMETOX bioactive compounds at 2xyh by Toxicities

Compound	Toxicities						Lipinski
	AMES	Oral Acute Toxicity	Carcinogenicity	Respiratory Toxicity	LC50FM	LC50DM	
Miraxanthin-V	---	---	---	++	2.886	3.451	Yes
n-Hexyl glucosinolate	---	--	---	+	3.764	4.726	Yes
Sinalbin	---	--	---	--	3.888	4.613	Yes
Donepezil	---	--	---	+++	5.338	6.367	Yes

Miraxanthin-V has the highest stability when compared to the other three compounds and native ligand. In molecular dynamics, RMSD represents the attachment of a protein to a compound. When the attachment is stable, the stable RMSD graph is generated [55][38]. The stability of attachment suggests that the compound may have a biological effect [38][46]. Figure 5 shows that Miraxanthin-V is more stable than Native Ligand and donepezil, with donepezil serving as the positive control in molecular simulations of Alzheimer's disease. Because of this phenomenon, Miraxanthin-V is a better drug candidate. The RMSF Miraxanthin-V profile is shown in Figure 6.

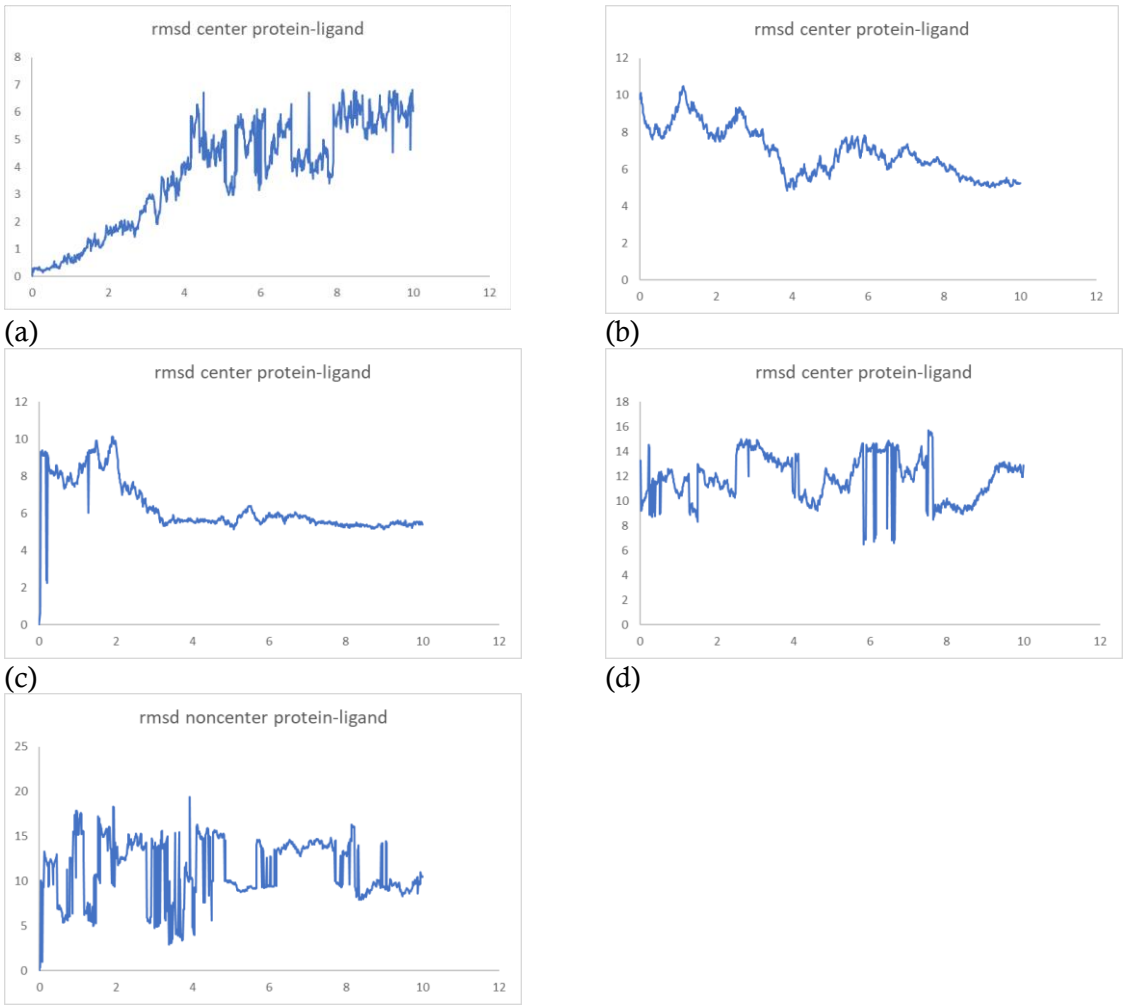

Figure 5. RMSD parameter 2xyh (a) Native Ligand, (b) Donepezil-Positive Control, (c) Miraxanthin-V, (d) Sinalbin, (e) N-hexyl Glucosinolate

The RMSF graph in Figure 6 corresponds to the amino acid residues in Table 4. Table 4 shows that good binding sites for amino acid residues are found near amino acid residue points 50s, 120, and 150-160. The consensus of amino acid residues is critical in properly handling the compound [40][33]. As a result, their stability is critical because if the amino acid residues cannot hold the compound perfectly, it will have a negative biological effect [56][27]. The amplitude graph of RMSF shows the stability of amino acid residues; the x-axis represents the number of amino acids, while the y-axis represents the RMSF. The presence of 5 hydrogen bonds in the Miraxanthin-V compound as a result

of docking indicates the binding affinity between protein and miraxanthin-v. The higher the affinity, the more hydrogen bonds there are. Thr62, Gly122, and Ser120 are the amino acid residues in Miraxanthin-V hydrogen bonds. Gly122 and Ser120 were proposed as the active sites because they are found in both the native ligand and the positive control (donepezil). Thr62 is involved in hydrogen bond formation. Furthermore, Miraxanthin-V is expected to be more effective than donepezil in the treatment of Alzheimer's disease.

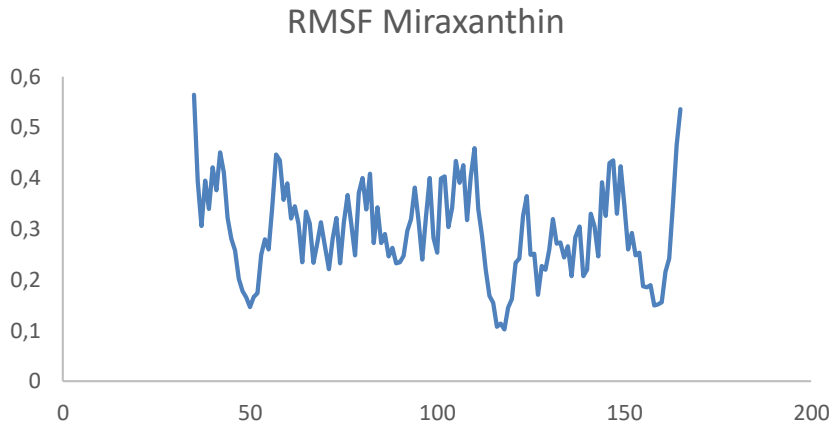

Figure 6. RMSF Miraxanthin-V

4. Conclusion

Miraxanthin-V is a bioactive compound found in herbal plants in Indonesia. Miraxanthin has a low toxicity value and meets all of the rules of the Lipinski Rule of Five, according to molecular simulations. Molecular docking and molecular dynamics result also revealed that Miraxanthin could bind stably to the apoptotic protein caspase3, 2xyh. As a result, miraxanthin is thought to be capable of inhibiting apoptosis in Alzheimer's patients. However, in order to ensure the effectiveness and pharmacological activity of the Miraxanthin compound, a wet lab test is required.

References

[1] Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer ' s Disease : *Molecules*, *25*(24), 5789.

[2] Centers for Disease Control and Prevention. (2018). *What is Alzheimer's Disease? | CDC*. https://www.cdc.gov/aging/aginginfo/alzheimers.htm%0Ahttps://www.cdc.gov/aging/aginginfo/alzheimers.htm%0Ahttps://www.cdc.gov/aging/aginginfo/alzheimers.htm#known%0Ahttps://www.cdc.gov/aging/aginginfo/alzheimers.htm%0Ahttps://www.cdc.gov/aging/aginginfo/a

[3] Chi, H., Chang, H. Y., & Sang, T. K. (2018). Neuronal cell death mechanisms in major neurodegenerative diseases. *International Journal of Molecular Sciences*, *19*(10)

[4] Lei, P., Ayton, S., & Bush, A. I. (2021). The essential elements of Alzheimer's disease. *Journal of Biological Chemistry*, *296*, 100105

[5] Sehar, U., Rawat, P., Reddy, A. P., Kopel, J., & Reddy, P. H. (2022). Amyloid Beta in Aging and Alzheimer's Disease. *International Journal of Molecular Sciences*, *23*(21).

[6] Erin R. HascupErnesto Solis Jr.Kevin N. Hascup. (2020). Alzheimer's Disease: The Link Between Amyloid-β and Neurovascular Dysfunction. *Journal of Alzheimer's Disease*, *76*(4), 1179–1198.

[7] Liu, J., Chang, L., Song, Y., Li, H., & Wu, Y. (2019). The role of NMDA receptors in Alzheimer's disease. *Frontiers in Neuroscience*, *13*(FEB), 1–22

[8] Singh, R., Letai, A., & Sarosiek, K. (2020). Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Definitions*, *20*(3), 175–193.

[9] Espinosa-Oliva, A. M., García-Revilla, J., Alonso-Bellido, I. M., & Burguillos, M. A. (2019). Brainiac Caspases: Beyond the Wall of Apoptosis. *Frontiers in Cellular Neuroscience*, *13*(November), 1–9.

[10] Ułamek-Kozioł, M., Czuczwar, S. J., Kocki, J., Januszewski, S., Bogucki, J., Bogucka-Kocka, A., & Pluta, R. (2019). Dysregulation of Autophagy, Mitophagy, and Apoptosis Genes in the CA3 Region of the Hippocampus in the Ischemic Model of Alzheimer's Disease in the Rat. *Journal of Alzheimer's Disease*, *72*(4), 1279–1286.

[11] Ghoneum, M. H., & El Sayed, N. S. (2021). Protective Effect of Biobran/MGN-3 against Sporadic Alzheimer's Disease Mouse Model: Possible Role of Oxidative Stress and Apoptotic Pathways. *Oxidative Medicine and Cellular Longevity*, *2021*

[12] Wang, R., & Reddy, P. H. (2018). Role of Glutamate Receptor. *J. Alzheimer Dis.*, *57*(4), 1041–1048.

[13] Cummings, J., Feldman, H. H., & Scheltens, P. (2019). *The " rights " of precision drug development for Alzheimer ' s disease*. *5*, 1–14.

[14] Li;, H. W. Y. Z. J. W. Y. (2018). Combining in vitro and in silico Approaches to Find New Candidate Drugs Targeting the Pathological Proteins Related to the Alzheimer's Disease. *Current Neuropharmacology*, *16*, 758–768.

[15] Song, Z., He, C., Yu, W., Yang, M., Li, Z., Li, P., Zhu, X., Xiao, C., & Cheng, S. (2022). Baicalin Attenuated A β 1-42-Induced Apoptosis in SH-SY5Y Cells by Inhibiting the Ras-ERK Signaling Pathway. *BioMed Research International*, *2022*.

[16] Weller, J., & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research*, *7*(0), 1–9.

[17] Li, J., Sun, M., Cui, X., & Li, C. (2022). Protective Effects of Flavonoids against Alzheimer's Disease: Pathological Hypothesis, Potential Targets, and Structure–Activity Relationship. *International Journal of Molecular Sciences*, *23*(17).

[18] Cummings, J. L., Tong, G., & Ballard, C. (2019). Treatment Combinations for Alzheimer's Disease: Current and Future Pharmacotherapy Options. *Journal of Alzheimer's Disease*, *67*(3), 779–794.

[19] Olivares, D., Deshpande, V. K., Shi, Y., Lahiri, D. K., Greig, N. H., Rogers, J. T., & Huang, X. (2012). N-Methyl D-Aspartate (NMDA) Receptor Antagonists and Memantine Treatment for Alzheimer's Disease, Vascular Dementia and Parkinson's Disease HHS Public Access. *Curr Alzheimer Res*, *9*(6), 746–758.

[20] Tiwari, S., Venkata, A., Kaushik, A., Adriana, Y., & Nair, M. (2019). Alzheimer ' s Disease Diagnostics And Therapeutics Market. *Int J Nanomedicine .*, *Jul 2019*(14), 5541–5554.

[21] Batool, M., Ahmad, B., & Choi, S. (2019). A structure-based drug discovery paradigm. *International Journal of Molecular Sciences*, *20*(11).

[22] Pengpid, S., & Peltzer, K. (2019). Use of traditional medicines and traditional practitioners by children in Indonesia: Findings from a national population survey in 2014-2015. *Journal of Multidisciplinary Healthcare*, *12*, 291–298.

[23] You, J. S., Li, C. Y., Chen, W., Wu, X. L., Huang, L. J., Li, R. K., Gao, F., Zhang, M. Y., Liu, H. L., & Qu, W. L. (2020). A network pharmacology-based study on Alzheimer disease prevention and treatment of Qiong Yu Gao. *BioData Mining*, *13*(1), 1–12.

[24] Widyowati, R., & Agil, M. (2018). Chemical constituents and bioactivities of several Indonesian plants typically used in jamu. *Chemical and Pharmaceutical Bulletin*, *66*(5), 506–518.

[25] Budiarti, M., Maruzy, A., Mujahid, R., Sari, A. N., Jokopriyambodo, W., Widayat, T., & Wahyono, S. (2020). The use of antimalarial plants as traditional treatment in Papua Island, Indonesia. *Heliyon*, *6*(12), e05562.

[26] Zhang, R., Zhu, X., Bai, H., & Ning, K. (2019). Network pharmacology databases for traditional Chinese medicine: Review and assessment. *Frontiers in Pharmacology*, *10*(February), 1–14.

[27] Azminah, A., Erlina, L., Radji, M., Mun'im, A., Syahdi, R. R., & Yanuar, A. (2019). In silico and in vitro identification of candidate SIRT1 activators from Indonesian medicinal plants compounds database. *Computational Biology and Chemistry*, *83*(June), 107096.

[28] Rifaai, R. A., Mokhemer, S. A., Saber, E. A., El-Aleem, S. A. A., & El-Tahawy, N. F. G. (2020). Neuroprotective effect of quercetin nanoparticles: A possible prophylactic and therapeutic role in alzheimer's disease. *Journal of Chemical Neuroanatomy*, *107*(April).

[29] Gregory, J., Vengalasetti, Y. V., Bredesen, D. E., & Rao, R. V. (2021). Neuroprotective herbs for the management of alzheimer's disease. *Biomolecules*, *11*(4), 1–19.

[30] Erlina, L., Paramita, R. I., Kusuma, W. A., Fadilah, F., Tedjo, A., Pratomo, I. P., Ramadhanti, N. S., Nasution, A. K., Surado, F. K., Fitriawan, A., Istiadi, K. A., & Yanuar, A. (2022). Virtual screening of Indonesian herbal compounds as COVID-19 supportive therapy: machine learning and pharmacophore modeling approaches. *BMC Complementary Medicine and Therapies*, *22*(1), 1–19.

[31] Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., Zheng, M., Chen, L., & Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B*, *10*(5), 766–788.

[32] Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, *20*(18).

[33] Hemalatha, G., Sivakumari, K., Rajesh, S., & K, S. D. (2020). In silico molecular docking studies of muricin J, muricin K and muricin L compound from A. muricata againts apoptotic proteins (caspase-3, caspase-9 and β-actin). *Innoriginal Originating Innovation*, *7*(5), 1–4.

[34] Lin, X., Li, X., & Lin, X. (2020). A review on applications of computational methods in drug screening and design. *Molecules*, *25*(6), 1–17.

[35] Patel, H., & Kukol, A. (2021). Integrating molecular modelling methods to advance influenza A virus drug discovery. *Drug Discovery Today*, *26*(2), 503–510.

[36] Ojo, O. A., Ojo, A. B., Okolie, C., Nwakama, M. A. C., Iyobhebhe, M., Evbuomwan, I. O., Nwonuma, C. O., Maimako, R. F., Adegboyega, A. E., Taiwo, O. A., Alsharif, K. F., & Batiha, G. E. S. (2021). Deciphering the interactions of bioactive compounds in selected traditional medicinal plants against alzheimer's diseases via pharmacophore modeling, auto-QSAR, and molecular docking approaches. *Molecules*, *26*(7).

[37] Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, *367*(6485), 1444–1448.

[38] Boittier, E. D., Tang, Y. Y., Buckley, M. E., Schuurs, Z. P., Richard, D. J., & Gandhi, N. S. (2020). Assessing molecular docking tools to guide targeted drug discovery of cd38 inhibitors. *International Journal of Molecular Sciences*, *21*(15), 1–19.

[39] Opo, F. A. D. M., Rahman, M. M., Ahammad, F., Ahmed, I., Bhuiyan, M. A., & Asiri, A. M. (2021). Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. *Scientific Reports*, *11*(1), 1–18.

[40] Jabir, N. R., Rehman, M. T., Alsolami, K., Shakil, S., Zughaibi, T. A., Alserihi, R. F., Khan, M. S., AlAjmi, M. F., & Tabrez, S. (2021). Concatenation of molecular docking and molecular

simulation of BACE-1, γ-secretase targeted ligands: in pursuit of Alzheimer's treatment. *Annals of Medicine*, *53*(1), 2332–2344.
[41] Torres, P. H. M., Sodero, A. C. R., Jofily, P., & Silva-Jr, F. P. (2019). Key topics in molecular docking for drug design. *International Journal of Molecular Sciences*, *20*(18), 1–29.
[42] Vyse, S., & Huang, P. H. (2019). Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer. *Signal Transduction and Targeted Therapy*, *4*(1), 1–10.
[43] Muhammed, M. T., & Aki-Yalcin, E. (2019). Homology modeling in drug discovery: Overview, current applications, and future perspectives. *Chemical Biology and Drug Design*, *93*(1), 12–20.
[44] *RCSB PDB - 2XYH_ Caspase-3_CAS60254719*.
[45] Syahdi, R. R., Iqbal, J. T., Munim, A., & Yanuar, A. (2019). HerbalDB 2.0: Optimization of construction of three-dimensional chemical compound structures to update Indonesian medicinal plant database. *Pharmacognosy Journal*, *11*(6), 1189–1194.
[46] Erlina, L., Paramita, R. I., Kusuma, W. A., Fadilah, F., Tedjo, A., Pratomo, I. P., Ramadhanti, N. S., Nasution, A. K., Surado, F. K., Fitriawan, A., Istiadi, K. A., & Yanuar, A. (2020). *Virtual Screening on Indonesian Herbal Compounds as COVID-19 SupportiveTherapy: Machine Learning and Pharmacophore Modeling Approaches*.
[47] Maia, E. H. B., Assis, L. C., de Oliveira, T. A., da Silva, A. M., & Taranto, A. G. (2020). Structure-Based Virtual Screening: From Classical to Artificial Intelligence. *Frontiers in Chemistry*, *8*(April).
[48] Garrett M. Morris. (2010). AutoDock Version 4.2 - User Guide. *Guide*, 1–49.
[49] *ChemAxon - Software Solutions and Services for Chemistry & Biology* (Vol. 2019, Issue February 4). (2019).
[50] Valencia, P. L., Sepúlveda, B., Gajardo, D., & Astudillo-Castro, C. (2020). Estimating the product inhibition constant from enzyme kinetic equations using the direct linear plot method in one-stage treatment. *Catalysts*, *10*(8), 1–10.
[51] Zhou, F., He, K., Guan, Y., Yang, X., Chen, Y., Sun, M., Qiu, X., Yan, F., Huang, H., Yao, L., Liu, B., & Huang, L. (2020). Network pharmacology-based strategy to investigate pharmacological mechanisms of Tinospora sinensis for treatment of Alzheimer's disease. *Journal of Ethnopharmacology*, *259*(May), 112940.
[52] de Ávila, M. B., & de Azevedo, W. F. (2018). Development of machine learning models to predict inhibition of 3-dehydroquinate dehydratase. *Chemical Biology and Drug Design*, *92*(2), 1468–1474.
[53] Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., & Cao, D. (2021). ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Research*, *49*(W1), W5–W14.
[54] Lemkul, J. (2019). From Proteins to Perturbed Hamiltonians: A Suite of Tutorials for the GROMACS-2018 Molecular Simulation Package [Article v1.0]. *Living Journal of Computational Molecular Science*, *1*(1), 1–53.
[55] Cui, Q., Zhang, Y. liang, Ma, Y. hui, Yu, H. yu, Zhao, X. zhe, Zhang, L. hui, Ge, S. qin, Zhang, G. wei, & Qin, X. de. (2020). A network pharmacology approach to investigate the mechanism of Shuxuening injection in the treatment of ischemic stroke. *Journal of Ethnopharmacology*, *257*, 112891.
[56] Zu, G., Sun, K., Li, L., Zu, X., Han, T., & Huang, H. (2021). Mechanism of quercetin therapeutic targets for Alzheimer disease and type 2 diabetes mellitus. *Scientific Reports*, *11*(1), 1–11.