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Potential of the vitexin compound in Kecombrang Kombucha Flowers (Etlingera elatior) as a candidate for antidiabetic, colon cancer and anti-inflammatory drugs based on bioinformatics analysis

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Abstract

Background: Discovery of new drugs is a major challenge for the future using AI. A combination of experimental and computational methods is a method best in discovering new drugs. Kecombrang flower kombucha contains vitexin that inhibits the compound α-glucosidase which is a potential compound as an antidiabetic because it can reduce blood sugar levels. Kecombrang also contains antioxidant and anti-inflammatory which is good for health and can suppress cancer growth.
Method: This study using Descriptive with In-Silico analysis using Bioinformatic tools such as Autodock Tools, Autodock Vina, Open Babel, Avogadro. This research is limited to the molecular docking stage. The 3-dimensional structure of the Vitexin compound downloaded from site https://pubchem.ncbi.nlm.nih.gov with pubchem code CID_5280441. The 3-dimensional structure taken is a co-crystal of α-glucosidase (PDB code: 2QMJ), Chk1 receptor (PDB ID: 2R0U), Cyclin (PDB ID: 6GUE), COX1 Receptor (PDB code: 6Y3C), and COX2 Receptor (PDB code: 5F19).
Results: The results showed that vitexin binds to α-glucosidase with ΔG value of -2.65 kcal/mol and the presence of bonds The hydrogen formed is residues VAL455 and SER456 and also binds to enzymes Chkl and Cyclin A. The value of ΔG binding at Chkl -5.75 kcal/mol, forming hydrogen bonds ARG A:74 & HIS A:73. On Cyclin A -6.19 kcal/mol, forming hydrogen bonds TYR B:271. COX1 receptor with the test ligand vitexin compound, was obtained Gibbs Energy (ΔG0)-8.2 kcal/mol for COX2 Gibbs Energy (ΔG0) -9.1 kcal/m.
Conclusion: Vitexin is predicted to have the potential to inhibit the enzyme α-glucosidase, Checkpoint Kinase 1 enzyme (Chk1) & Cyclin A in suppressing cancer cell growth. Vitexin is also predicted to inhibit COX1 and COX2 enzymes as anti-inflammatory.

Keywords: Vitexin; Kombucha Kecombrang Flowers; Candidate; Anti-diabetic; Anti-colon cancer; Anti-inflammatory

INTRODUCTION

Kombucha is a beverage product resulting from the fermentation of a solution of tea and sugar by adding a kombucha microbial starter, namely Acetobacter xylinum and several types of yeast or kombucha fungus. Kombucha fermentation time ranges from 8-12 days at a temperature of 18-20°C, while at higher temperatures the fermentation takes less time. The organic acid content in kombucha is acetic acid which acts as an antibacterial. In this research, the basic ingredient for kombucha was made from kecombrang flowers (Etlingera elatior). Apart from that (1) stated that butterfly pea flower kombucha has anticancer properties(1).

Kecombrang has been reported to have pharmacological activities, including: as anticancer, antiproliferative and cytotoxic. Combrang flowers (Etlingera elatior) contain large amounts of phenolics and flavonoids such as gallic acid, caffeic acid, quercetin, luteolin and myricetin which can inhibit the growth of breast cancer cells. Vitexin is a compound that is included in the flavonoid group. This compound is often found in kombucha. The results of research by (2) found that butterfly pea flower kombucha has anti-IgE(2).

Diabetes mellitus is a disease caused by a relative or absolute lack of insulin, or if the body cannot effectively use the insulin it produces over time, it can cause damage to the heart, blood vessels, eyes, kidneys and nerves. α-glucosidase is an enzyme that plays a role in the process of carbohydrate metabolism which is located at the edge of the surface of small intestinal cells, as well as the process of glycoproteins and glycolipids. Glucosidase works by breaking down carbohydrates into glucose in the human small intestine. Compounds that can inhibit glucosidase activity are compounds that have the potential to act as antidiabetics, because they can reduce blood sugar levels. Problems related to insulin therapy arise from repeated daily injections and blood sampling that cause pain or trauma or damage to the skin making it difficult to achieve an optimal treatment regimen.

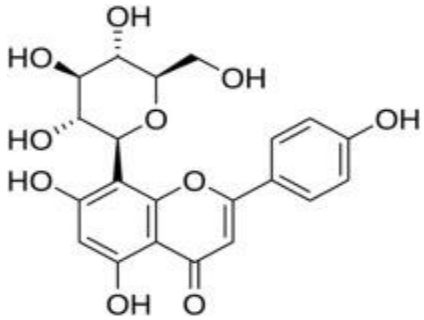


Figure 1. Structure of Vitexin Compound
Source: Pubchem. Vitexin. [on line]. 2024 [cited 2024 Jan 17]; Available from:
URL: https://pubchem.ncbi.nlm.nih.gov

Cancer is a disease that poses a threat to human health. According to Smith et al., (2019) regarding the American Cancer Society (2019) cancer is a disease characterized by the growth and spread of abnormal cells that cannot be controlled, if this continues to happen and is not controlled it will cause death(3). Growth and spread of abnormal cells. If it occurs in the cells that make up the large intestine to the rectum, it is called colorectal cancer (4),(5) . Treatment therapy for colorectal cancer includes radiotherapy, chemotherapy and surgery. One of the chemotherapy drugs is 5-Fluorouracil(5-FU), a drug. It is used as a positive control or standard comparative ligand that can be used to help improve the survival of patients with various types of cancer(5).

Cyclooxygenases (COXs) are key enzymes in the conversion of arachidonic acid to prostaglandins. There are two isoforms of COX. COX-1 is a constitutive enzyme found in most normal tissues and is responsible for local prostaglandin synthesis. The cyclooxygenase (COX) receptor is a dual function binding receptor on the membrane which plays a role in catalyzing two important stages in the formation of prostanoids, namely cyclooxygenation and peroxidation. Currently, many anti-inflammatory compounds are found from natural ingredients. However, it is not yet known which compounds selectively inhibit COX-1 and COX-2 receptors. In silico research on vitexin in butterfly pea flower kombucha was carried out by Fadillah et al., (2024) which has been proven to be a source of antioxidants and anticancer(6). Saputri et al., (2024) where the vitexin compound in kecombrang flower kombucha can be predicted as a candidate antidiabetic drug (7),(8).

METHOD

The type of research used was in-depth descriptive research in silico of the Vitexin compound of kecombrang kombucha flowers (Etlingera elatior) using hardware with specifications Intel (R) Core (TM) i7 2670QM @ 2.20GHz 2.20GHz RAM 8.00 GB. The programs used are Discovery Visualizer Studio 2021, Autodock Tools, Autodock Vina, Open Babel, Avogadro. The 3-dimensional structure of the Vitexin compound comes from the site https://pubchem.ncbi.nlm.nih.gov with the pubchem code CID_5280441 3-Dimensional Protein Structure Protein structures involved in diabetes pathogenesis were taken from the database, namely PDB (Protein Data Bank) in co-crystal form (PDB.2QMJ), while colon cancer were taken from Chk1 receptor (PDB ID: 2R0U), Cyclin (PDB ID: 6GUE) and Apoptosis Regulator Bcl-2 (PDB ID:4LXD),and for anti-inflamatory were taken

from COX1 Receptor (PDB code: 6Y3C), and COX2 Receptor (PDB code: 5F19).

RESULTS

Molecular docking validation was carried out by re-docking the acarbose ligand with the α glucosidase receptor (PDB code: 2QMJ) which had been prepared with Grid Box. The positions used are x = 0.803, y = -0.102, z = 0.309, while the dimensions x = 32, y = 26, z = 20 with a spacing of 0.375 Å. This validation was carried out to determine the conformational similarity between the original crystallographic ligand and the original ligand resulting from docking optimization (9). Visualization of the validation results shows a comparison of the position of the receptors and original ligand resulting from docking.

Table 1. The validation results

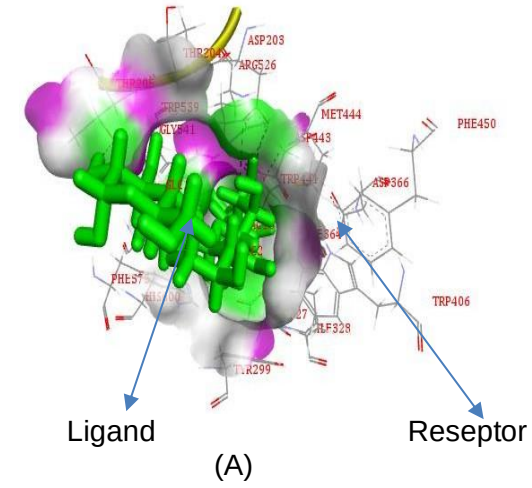
Recep-tor	Cluster	Confor mation	Binding energy	Reference RMSD
2QMJ	1	16	-9,06 kcal/mol	1,564 A
2R0U	1	2	-10.81 kcal/mol	0.944 Å
6GUE	1	1	-10.27 kcal/mol	0.902 Å
COX-1	1	1	- 10,7 kcal/mol	0.0 Å
COX-2	1	1	-9,1	0.0 Å

The validation results in the table (Table 1) show an RMSD value of 1.564 A which has a value < 2 A. RMSD is the value used to determine whether the prediction are successful and important to validation of the docking program. In general, value RMSD is said to be good if ≤ 2 A. The bigger it is deviation, the greater the error on the prediction of ligand interactions with proteins.

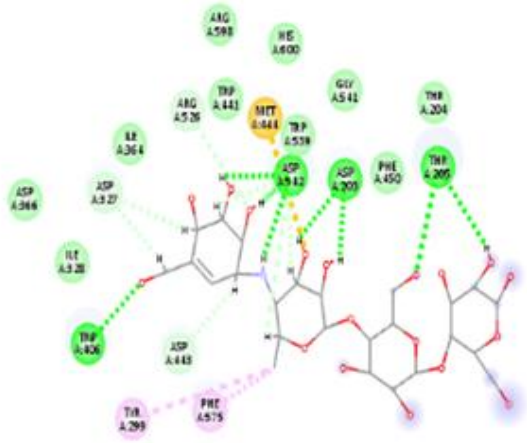
Table 2. Molecular Docking Results on the α-glucosidase receptor with vitexin

	Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num In Cluster
2QMJ	1	-2.65	31	-2.32	41
	2	-2.59	33	-2.52	59

The visualization results in table 2 show binding information between the ligand and the α-glucosidase receptor, namely Vitexin which is a ligand. The analysis results in table 2. show that the lower the anchor is repeatedly, the more negative or decreasing the binding affinity value is. (10) Vitexin is predicted to have the ability to inhibit the α-glucosidase enzyme which can reduce blood sugar levels.



(A)



(B)

Figure 1. 2D (a) and 3D (b) visualization of acarbose ligand interactions on the α- glucosidase receptor.

The interactions that occur in Acarbose (Figure 1) with the α-glucosidase receptor show that there are two types of interactions that occur, namely hydrophobic interactions and hydrogen interactions with varying interaction distances, different atoms or functional groups that bind to the receptor,

and interacting amino acid residues varies too. In the Vitexin ligand (Figure. 2), it can be seen that there are 2 types of bonds that occur, namely hydrophobic bonds and hydrogen bonds with different interaction distances, atoms or functional groups and varying interaction distances. In the interactions that occur between ligands and receptors, there are only 2 types of interactions, namely hydrogen interactions and various hydrophobic interactions.

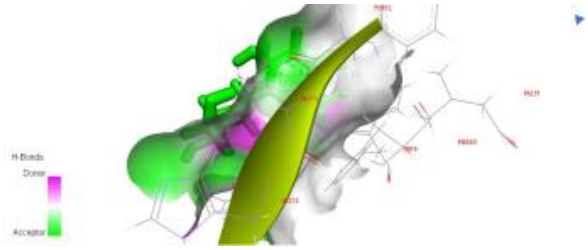
The visualization results in figure 2 (b) show binding information between the ligand and the α-glucosidase receptor, namely 5-Fluorouracil (5-FU) as a comparative ligand and Vitexsin which is a ligand with a higher ΔG value than the ΔG value of 5-Fluorouracil (5-FU).

Validation of the macromolecular docking method was carried out on each target protein used, namely Checkpoint Kinase 1 (Chk1) (PDB ID: 2R0U) and CyclinA (PDB ID: 6GUE). The molecular docking process was carried out using the Autodock Vina application and the MGL Tool (Autodock Tool). The results of molecular docking of kecombrang flower kombucha can be seen in table 2 below:

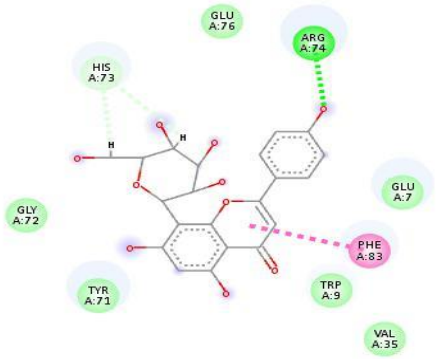
Table 2. Molecular Docking Results on the colon cancer receptors with vitexin

Receptor	Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num In Cluster
2R0U	1	-5.75	90	-5.52	51
	2	-5.69	88	-5.59	5
	3	-5.38	27	-5.32	44
6GUE	1	-6.19	63	-5.52	31
	2	-5.72	57	-5.50	42
	3	-5.41	42	-5.26	4
	4	-5.32	50	-5.17	13
	5	-4.96	14	-4.92	3
	6	-4.89	17	-4.86	7

Based on the results of docking the vitexin compound with 2R0U, 9 binding amino acid residues were obtained, namely GLY A:72; TYR A:71; TRP A:9; VAL A:35; GLU A:7; GLU A:76; ARG A:74; HIS A:73; PHE A:83. The interaction formed between the reference ligand, namely 5-Fluorouracil(5-FU) and 2R0U, forms hydrogen bonds at the amino acid residues ARG A:74 & HIS A:73 (Figure 4.3). The interaction formed between the comparison ligand, namely 5-Fluorouracil (5-FU) and 6GUE, forms a hydrogen bond at the amino acid residue TYR B:271 (Figure 2).



A)



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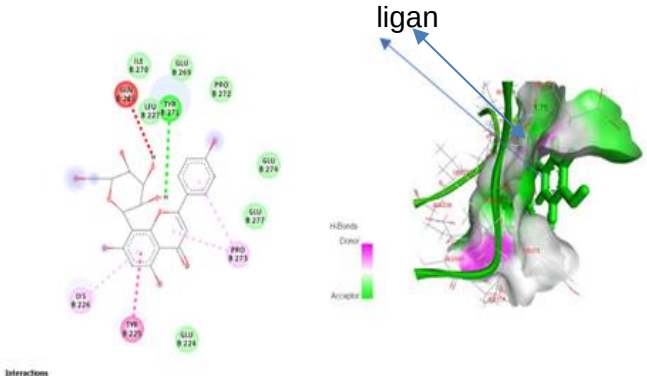

(C)

Figure 2. 2D (a) and 3D (b) Visualization of Vitexin Ligand on the 2R0U receptor, (c) 2D and 3D Visualization of Vitexin Ligand on the 6GUE receptor

Validation of the macromolecular docking method was carried out on each target protein used, namely COX1 (ID code: 6Y3C) and COX2 (ID code: 5F19). The molecular docking process was carried out using the Autodock Vina application and MGL Tools (Autodock Tools). The results of molecular re-docking of kecombrang flower kombucha can be seen in table 3 below:

Table 3. Molecular Docking Results on the anti-inflammatory receptors with vitexin

Receptor	Cluster Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num In Cluster
6Y3C	1	-8.20	90	-8.20	51
	2	-8.29	88	-8.29	5
	3	-8.22	27	-8.22	44
5F19	1	-9.10	63	-9.10	31
	2	-9.12	57	-9.12	42
	3	-9.16	42	-9.16	4

The results in table 3 of docking the COX1 receptor with the vitexin test ligand compound, obtained an Energy Binding Affinity (ΔG) of -8.2 kcal/mol. This value is a low value compared to other structural confirmations. Energy Binding Affinity defines the strength of the bond formed between the receptor and the ligand. Then in table 3 for the docking results of the COX2 receptor with the vitexin test ligand compound, the Gibbs Energy (ΔG0) is -9.1 kcal/mol. Based on the data collected, it is stated that COX2 has the strongest energy binding affinity compared to COX1. The stability of a compound can be seen from its low binding energy.

Based on Figure 3, a 2D visualization of the docking results of the vitexin ligand molecule with the COX-1 (6Y3C) enzyme receptor, several bonds can be seen from each compound, namely van der Waals bonds, conventional hydrogen bonds and carbon hydrogen bonds. In the vitexin compound, van der Waals bonds are formed at the amino acid residues ILE444, ALA202, THR206, GLU454. Hydrogen bonds are formed at amino acid residues TYR385, and GLN203.

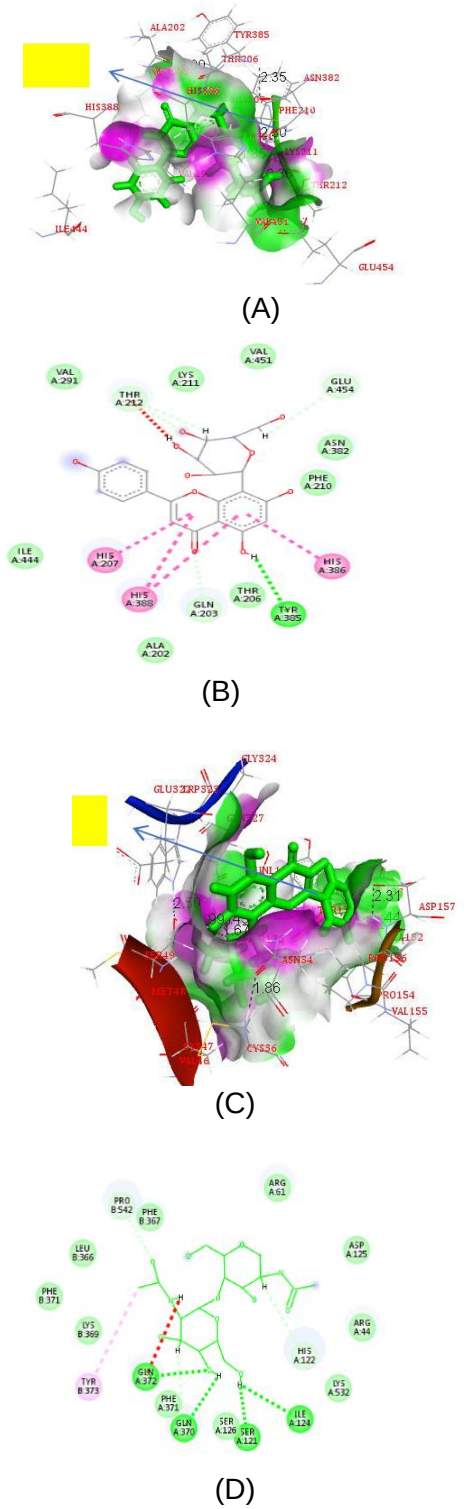

Figure 3. 2D (a) and 3D (b) Visualization of Vitexin Ligand on the 6Y3C receptor, (c) 2D and 3D Visualization of Vitexin Ligand on the 5F19 receptor

Meanwhile, in the 2D visualization results in Figure 4.6, the results of docking

the vitexin ligand molecule with the COX-2 enzyme receptor (5F19) show that several bonds are formed from each compound, namely van der Waals bonds and conventional hydrogen bonds. and carbon hydrogen bonds. In vitexin compounds, van der Waals bonds are formed at amino acid residues ASP133, TYR134, PRO154, VAL155, PRO158, CYS36, TYR136, MET48, ASN34, VAL46, TRP323, GLU322, GLY324. Hydrogen bonds are formed at amino acid residues ALA132, GLN327, SER49, GLY135, and CYS4.

DISCUSSION

In the current era of artificial intelligence, research that is developing is genomics and preteomics. In this case, the discovery of new drugs is a future challenge, especially the use of AI. Several methods or ways to search for new drug candidates such as X-Ray Crystallography, Spectroscopy, NMR, High-throughput screening. Combinatorial chemistry and using computers. The combination of experimental and computational methods is the best method for discovering new drugs, because computing and experimentation can complement each other. The use of computational methods can reduce the use of test animals and chemicals used (11). Ligand-Based Drug Design (LBDD) is drug design based on a known ligand but unknown receptor structure. Meanwhile, Structure Based Drug Design (SBDD) is drug design based on the structure of the target receptor and uses information from the target receptor protein and uses information from the target protein to find the active site of the protein that binds to the drug compound. or ligand.(12)

The three-dimensional shape of biomolecules such as proteins, DNA and RNA is very important because it can help analyze the specific targets of a disease. Utilizing this three-dimensional shape information can be used to search for new drug candidates using the Structure Based Drug Design (SBDD) approach. This method utilizes information from the three-dimensional structure of the protein to see the part or active site that binds to the drug. The SBDD method is influenced by three-dimensional protein information analyzed using X-Ray Crystallography and NMR. From three-dimensional data, the active site of a protein can be identified so that drugs can be designed that have biological activity. The target protein structure can be modeled using the Homology modeling approach which can be accessed at www.rscb.org.(13)

Isolation of proteins with test ligands is the initial process in the development of new drugs. Interactions between proteins and ligands influence biological processes in the body. Isolation of proteins and ligands is also very important because the drug effect will occur if there is a bond between the protein and the receptor. With the SBDD computational approach, compounds binding to their biological targets can be visualized. The information obtained is in the form of molecular interactions such as hydrogen bonds, salt bridges, repulsive forces and attractive van Der Waals forces.(14)(15)

The validation results listed in tables 1 to three have explained that the docking method used has quite high accuracy. This was proven by changes in binding with a percentage of less than 2%, thus indicating that in this study the docking method could be trusted in predicting ligand and receptor interactions. Ideal validation criteria include having an initial binding value and after docking less than 5% through consistent repetition, namely 3 times (16), (17)

The most popular method in drug design is molecular docking, which is used to see the best shape or pose of receptors and ligands. Docking is also based on the lock and key principle which is Emil Fischer's concept discovered in 1894. Docking involves predicting the shape or conformation of the ligand and the bond orientation. This method is able to place the ligand in the active site (binding site) with an accurate conformation so that it can interact with the receptor. Apart from being based on lock and key docking, it also has the principle of ulated fit, macromolecular forms and small molecular forms can enter and bond.(18)(19)

Docking algorithms are also able to predict the binding of ligands, small molecules, peptides and proteins. The results obtained include orientation, conformation and interactions between proteins. Docking can be done with a computer with minimum specifications such as a laptop. With docking, results are obtained quickly, but there is still a lot of data whose results do not match when compared with experimental results. Protein ligand docking simulation is a computational methodology that primarily attempts to find the position of a ligand within a protein binding site. Docking is able to predict ligand-protein interactions which are very important in biochemical processes. The docking results between ligand and protein can be visualized using the Discovery Studio, VMD, Pymol, etc. applications so that the optimal conformation form can then be identified (20).

This research was carried out in silico using a molecular docking method between diabetes drug receptors and chemical compounds contained in kecombrang flower kombucha. In silico testing requires a validated protocol to be able to identify compounds as receptor ligands (21). This research aims to obtain predictions of the binding of the Vitexin compound ligand to the maltase-glucoamylase receptor using in silico analysis and obtain RMSD (Root Mean Square Deviation) values from good ligand-protein conformations to be used as diabetes mellitus drug candidates. The compound in kecombrang flower kombucha tested in this study was vitexin. This compound was chosen because the vitexin compound has activity in inhibiting the α-glucosidase enzyme that causes diabetes. Meanwhile, the comparison compound used is acarbose because it is known that this compound is generally used in diabetes mellitus therapy(9). (18)

The compound used as a ligand in this research is vitexin in combrang kombucha flowers which meets 5 Lipinski parameters including molecular weight < 500, logarithmic octanol partition coefficient (LogP) < 5, hydrogen bond donor (HBD) < 5, hydrogen bond acceptor (HBA)<10, and no more than two errors/violations.(9)

Docking activity can be seen in Table 1. The binding affinity of a drug is best if it has the lowest or more negative binding energy value. This binding results in the formation of more interactions between the receptor and the ligand due to more free energy. released. As a result, the desired target is easier to reach than ligands with lower negative values. Bond affinity or bond energy value between a protein and a ligand. Binding affinity indicates the ability of a protein to bind to its ligand at a specific position. The α-glucosidase and vitexin enzymes have a binding affinity of -2.65 Kcal/mol. The α-glucosidase enzyme ligand (vitexin) interacts with the active site on 6 amino acid residues, while the natural ligand, namely acarbose, shows a binding affinity of -9.06 Kcal/mol and has an active site on 20 amino acid residues. Based on the visualization results in 2D form, several bonds are formed from each compound, namely van der Waals bonds, conventional hydrogen bonds, and carbon hydrogen bonds. In the vitexin compound, van der Waals bonds are formed at the amino acid residues GLN409, VAL451, ASP452, and GLY457. Hydrogen bonds are formed at amino acid residues VAL455 and SER456.

Checkpoint Kinase 1 (2R0U) and vitexin have a binding affinity of -5.75 Kcal/mol. The Checkpoint Kinase 1 enzyme ligand (vitexin) interacts with the active site on 9 amino acid residues, while the natural ligand, namely 5-Fluorouracil(5-FU) shows a binding affinity of -10.81 Kcal/mol and has an active site on 2 amino acid residues. (ARG A:74 & HIS A:73). Based on the results of docking the vitexin compound with 2R0U, 9 binding amino acid residues were obtained, namely GLY A:72; TYR A:71; TRP A:9; VAL A:35; GLU A:7; GLU A:76; ARG A:74; HIS A:73; PHE A:83. Cyclin A (6GUE) and vitexin have a binding affinity of -6.19 Kcal/mol. The Checkpoint Kinase 1 enzyme ligand (vitexin) interacts with the active site at 2 amino acid residues, while the natural ligand 5-Fluorouracil(5-FU) shows a binding affinity of -10.27 Kcal/mol and has an active site at 2

amino acid residues (ARG A:74 & HIS A:73). For the interaction formed between the comparison ligand, namely 5-Fluorouracil(5-FU) and 6GUE, it forms a hydrogen bond at the amino acid residue TYR B:271.

The results in table 2 of docking the COX1 receptor with the vitexin test ligand compound, obtained an Energy Binding Affinity (ΔG) of -8.2 kcal/mol. This value is a low value compared to other structural confirmations. Binding Affinity Energy defines the strength of the bond formed between the receptor and the ligand. Then in table 4.3 for the docking results of the COX2 receptor with the vitexin test ligand compound, the Gibbs Energy (ΔG0) is -9.1 kcal/mol. Based on the data collected, it is stated that COX2 has the strongest energy binding affinity compared to COX1. The stability of a compound can be seen from its low binding energy (22).

Based on Figure 4.1, a 2D visualization of the docking results of the vitexin ligand molecule with the COX-1 (6Y3C) enzyme receptor, several bonds can be seen from each compound, namely van der Waals bonds, conventional hydrogen bonds and carbon hydrogen bonds. In the vitexin compound, van der Waals bonds are formed at the amino acid residues ILE444, ALA202, THR206, GLU454. Hydrogen bonds are formed at amino acid residues TYR385, and GLN203. Meanwhile, in the 2D visualization results in Figure 4.2, the results of docking the vitexin ligand molecule with the COX-2 enzyme receptor (5F19) show that several bonds are formed from each compound, namely van der Waals bonds and conventional hydrogen bonds. and carbon hydrogen bonds. In vitexin compounds, van der Waals bonds are formed at amino acid residues ASP133, TYR134, PRO154, VAL155, PRO158, CYS36, TYR136, MET48, ASN34, VAL46, TRP323, GLU322, GLY324. Hydrogen bonds are formed at amino acid residues ALA132, GLN327, SER49, GLY135, and CYS47 (23).

Factors that influence the success of molecular docking are hydrophobic and hydrogen interactions which also contribute to the free energy value between the ligand-receptor. The factors that determine the ΔGbinding value are not only the number of interactions and the similarity of residues to the control ligand or comparison ligand. The ΔGbinding value is influenced by various factors, for example electrostatic interactions, lipophilicity/hydrophobicity, shape similarity, as well as other factors such as entropy and conformational changes. Hydrophobic interactions can also sometimes increase affinity more than hydrogen bonds (11),(24).

The cases discussed in this research are degenerative diseases such as diabetes mellitus, colon cancer and inflammation. In the future, this can also be done in cases of infectious diseases such as bacteria, fungi or viruses. Meanwhile, to prove it, bioassay methods such as in vitro and in vivo need to be carried out (25).(23)

CONCLUSIONS

This can be concluded that vitexin is predicted to inhibit the α-glucosidase enzyme which can lowers blood glucose levels, inhibits enzymes *Checkpoint Kinase 1* (Chk1) & Cyclin A in suppressing cell growth cancer and inhibits COX-1 and COX-2 enzymes in inflammatory reactions.

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