Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 11(1), 49-58, 2023

Study of Anthocyanin Molecule Blocking as Anti-Hypertensive through the Pathway of the Renin-Angiotensin-Aldosterone System (RAAS)

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Received: January 2023
Received in revised: February 2023
Accepted: May 2023
Available online: May 2023

Abstract

Anthocyanins are flavonoid-derived compounds that can reduce blood pressure. This study aims to determine the affinity value of the compound to bind to Angiotensin Converting Enzyme (ACE) and bind to Angiotensin II type 1 Receptor (AT1R) and to determine the distance and shape of the bond that occurs. The results of anthocyanin-derived compounds Delphinidin, Petunidin, Malvidin, Cyanidin, Peonidin, and Pelargonidin have anti-hypertensive potential through the Renin Angiotensin Aldosterone (RAAS) pathway based on molecular docking calculations. The affinity value of each, against Angiotensin Converting Enzyme (ACE) -7.7; -7.8; -7.7; -7.7; -7.8, and -7.7, the best affinity value in anthocyanin-derived compounds is shown in the Malvidin test compound which has three types of hydrogen bonds at a distance of ± 2 Å (ASP377, TYR520, ASP415) and has 1 type of bond that is the same as the lisinopril control (TYR520). While the affinity value to Angiotensin Receptor (AT1R) is -7.7; -7.7; -7.8; -7.7; -7.8, and -7.6, respectively, the best affinity value is shown in the Malvidin test ligand compound of -7.8 kcal/mol which has four types of hydrogen bonds ± 2 Å distance (TYR92, SER105, ARG167, TRP84) and has one kind of bond in common with lisinopril control (TYR520).

Keywords: Molecular docking, hypertension, renin-angiotensin, anthocyanin.

INTRODUCTION

Hypertension is a disorder of the circulatory system characterized by increased blood pressure. Hypertensive disease is included in the silent killer because it can appear without being known by the sufferer before checking their blood pressure (Smeltzer & Bare, 2013). Based on Basic Health Research (Riskesdas) on the prevalence of hypertension in Indonesia, with a population of around 260 million with a presentation of experiencing hypertension of 25.8% in 2013, it increased to 34.1% in 2018 (Wycidalesma & Yuswantina, 2021). Hypertension is expected to increase by around 80% by 2025, especially in developing countries (Herawati & Saptarini, 2020).

The hypertension mechanism involves the kidney's renal Angiotensin Aldosterone system (RAAS). Angiotensin Converting Enzyme (ACE) and Angiotensin II type 1 Receptor (AT1R) are proteins responsible for renal functions such as sodium resorption and dilation of blood vessels that affect blood pressure in the body (Williamson et al., 2017). Captopril, lisinopril, losartan, and Olmesartan, are Angiotensin Receptor Blocker (ARB) class drugs usually used by the public to treat hypertension. One of the ARB class drugs works by inhibiting Angiotensin II Type 1 (AT1) receptors which are found in the heart, blood vessels, and kidneys, thus helping to relax blood vessels and arteries to lower blood pressure and make it easier for the heart to pump blood Pressure reduction and dilation of blood vessels can be assisted by several classes of Flavonoid compounds. Anthocyanins are one of the Flavonoid groups; Anthocyanins have three carbon atoms bound by an oxygen atom to connect two aromatic benzene rings (C_6H_6) with a carbon skeleton ($C_6C_3C_6$).

Chemically anthocyanins include derivatives of a single aromatic structure with differences based on bonds with aromatic rings, thus forming several derivatives, including Cyanidin, Petunidin, delphinidin, Peonidin, Malvidin, and Pelargonidin (Zhang et al., 2015). Anthocyanins have 15 carbon atoms (C_{15}) outside of their substitution groups, where substitution groups are formed from pigments by the addition or subtraction of hydroxyl groups (Kamiloglu et al., 2015)

Hydroxyl groups play a role in the interaction by donating electrons to stabilize free radicals and inhibit enzymes and elements involved in free radical regeneration (Kumar & Pandey, 2013). Hydrogen in the hydroxyl group can form bonds with target receptors where hydrogen has a large electronegativity between atoms, and hydrogen bonding power is weaker than ionic bonds (Desaphy et al., 2013). The structure of the compound and the bonding mechanism between the drug compound and the receptor or target protein can be assessed and identified using the Molecular docking method or computational chemistry, often used in developing new drug compounds. This statement is because the computational chemistry method offers fast processing time at a low cost. Computational chemistry is assisted by computer devices to perform calculations, simulations, and geometry activities of a chemical compound against a receptor or target protein so that the affinity value or the value of the drug's ability to bind to the receptor or target protein is obtained (La Kilo et al., 2019)

Based on several studies, anthocyanins have the potential to inhibit Angiotensin Converting Enzyme (ACE) activity and bind to Angiotensin II type 1 Receptor (AT1R) (Khoo et al., 2018; López-Fernández-Sobrino et al., 2021). In a study by Ojeda Deynaria, anthocyanins contained in the flower petals of the Rosella plant (Hibiscus sabdariffa) can reduce blood pressure by inhibiting the activity of the Angiotensin Converting Enzyme (ACE) enzyme. Other studies have also shown that fruits such as blueberries, red berries, and elderberries contain very high anthocyanins, lowering blood pressure by providing a vasodilating effect on blood vessels (Mudnic et al., 2012; Wijianto et al., 2020). The purpose of this study was to determine the affinity value or the ability of anthocyanin-derived compounds to bind to the Angiotensin Converting Enzyme (ACE) receptor or protein and bind to Angiotensin II type 1 Receptor (AT1R) and to determine the distance and shape of the bond that occurs between each compound and the target receptor.

METHODOLOGY

Hardware

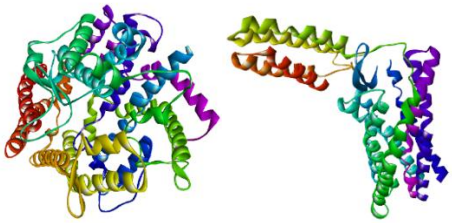
The device used in this research is a computer with Intel i3-9100F Processor specifications, 320GB SSD, 8GB DDR4 Ram, and GTX 1030 GPU.

Software

The applications used in this research are Discorverystudio 2016 Client, AutoDockTool (Version 1.5.6), Open Babel, Pymol (Version 2.0), Vina Wizard, PyRx, ChemOffice 2D (Version 19.0) and ChemOffice 3D (Version 19.0).

Materials

The materials used in the study were three-dimensional structures of anthocyanin-derived compounds, namely cyanidin, petunidin, delphinidin, peonidin, Malvidin, and pelargonidin (Table 1). The positive controls used were the drugs lisinopril, captopril, losartan, and Olmesartan in "sdf" format. The three-dimensional structures of Angiotensin Converting Enzyme (ACE) (PDB ID: 1086) and Angiotensin Receptor (AT1) (PDB ID: 4ZUD) proteins (Figure1), as well as their respective natural ligands, were downloaded from Protein Data Bank (http://www.rcsb.org/pdb/) in "pdb".



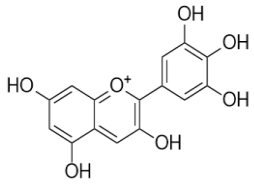
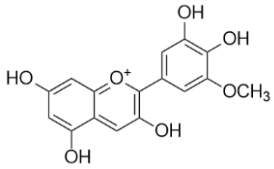
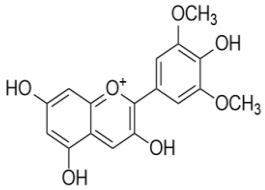
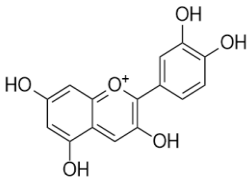
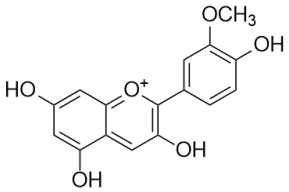
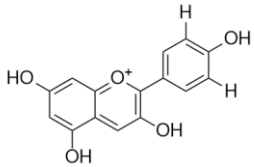
(ACE) (AT1)

Figure 1. Structure protein kristalin protein angiotensin-I converting enzyme (ACE) dan angiotensin reseptor (AT1)

Ligand Preparation

Anthocyanin-derived compounds and positive controls were obtained by drawing using the ChemOffice computer program (version 19.0). This process is to get information about the two-dimensional structure, the value of the log conversion of the Octanol-water partition (LogP), and molecular weight (BM). The compound that has been made is then converted to see the stereochemistry of the compound, and the most stable form of stereochemistry is arranged using the minimum energy tool in the Chem3D program (Version 19.0) so that the most stable form of compound structure is obtained in binding to the target receptor (Muttaqin, 2019).

Table 1 Two-dimensional structure of anthocyanin derivatives

Compound	Chemical Structure
Delphinidin	
Petunidin	
Malvidin	
Cyanidin	
Peonidin	
Pelargonidin	

Protein Preparation

Angiotensin Converting Enzyme (ACE) protein with code (PDB ID: 1086) and Angiotensin Receptor (AT1) with code (PDB ID: 1086) were downloaded from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) in "pdb" format. The preparation uses the AutoDockTool program (Version1.5.6) to remove water residues, remove the side of the atomic chain in the protein data, where the protein is specialized only polar by first doing geometry optimization and energy minimization, and changing the protein previously in the "pdb" file format to the "pdbqt" format. The change in protein file format is accompanied by adding hydrogen molecules. Determine the coordinates of the side location based on one of the built-in test ligands of the protein known to be the coordinates of the active side or the side of the protein that can bind (La Kilo et al., 2019).

Native Ligand Preparation

Native ligand preparation was using the DiscoveryStudio 2016 program. This preparation aims to obtain one of the natural ligands of Angiotensin Converting Enzyme (ACE) and Angiotensin Receptor (AT1) proteins downloaded from the protein data bank by separating the receptor and all molecules or compounds that are not used to leave one small molecule or ligand which is then used as a validation method (Muttaqin, 2019).

Lipinski's Rule of Five.

Lipinski's law, better known as Lipinski's law of five, is used to determine the hydrophilic-lipophilic nature of a compound. Lipinski has analyzed 2,245 drugs and concluded that the compound would be challenging to absorb. Its permeability is low if the molecular weight is more significant than 500, the log value of the octanol/water partition coefficient (log p) is more effective, has an acceptor H-bond, which is expressed by the number of O-H and N-H groups, more significant than five and has an acceptor H-bond, which is defined by the number of O and N atoms, greater than 10 (Hardjono, 2016).

Grid Box

Determining the grid box is one form of the parameter to specify the active side of the protein based on the box's location, which is determined based on the grid box size using the AutoDock Tool program (Version 1.5.6), where the results obtained

in the form of x, y, and z coordinates and the size of the grid box are used as a reference or location where the docking process will occur on the target protein (Kelutur, 2022; Mulyati & Panjaitan, 2021).

Converting Enzyme (ACE) Protein, Lisinopril, and Angiotensin Receptor (AT1), namely Olmesartan. Furthermore, docking results were visualized to see the active side of the protein that binds to the test ligand, the type of bond, and the distance between

Table 2 Chemical Physics Properties of Ligands

Name Compound	Formula Molecules	Weight Molecules	Log P	H bond donor	Acceptor H-bond
Delphinidin	$C_{15}H_{11}O_7$	303.246	2.6145	6	6
Petunidin	$C_{16}H_{13}O_7^+$	317.273	2.9175	5	6
Malvidin	$C_{17}H_{15}O_7^+$	331.08	3.2205	4	6
Cyanidin	$C_{15}H_{11}O_6^+$	287.06	2.9089	5	5
Peonidin	$C_{16}H_{13}O_6^+$	301.07	3.2119	4	5
Pelargonidin	$C_{15}H_{11}O_5^+$	271.06	3.2033	4	4
Losartan	$C_{22}H_{23}ClN_6O$	422.16	4.2668	2	6
Olmesartan	$C_{24}H_{26}N_6O_3$	446.21	3.6566	3	7
Lisinopril	$C_{21}H_{31}N_3O_5$	405.23	1.2352	4	5
Captropil	$C_9H_{15}NO_3S$	217.08	0.6279	2	3

Molecular Docking Process

When studying molecular docking, the intermolecular interactions of ligands and proteins can be analyzed (Maahury & Allo, 2021). The molecular docking process of the six anthocyanin-derived compounds against Angiotensin Converting Enzyme (ACE) and Angiotensin Receptor (AT1) proteins was carried out using the Vina Wizard program integrated into the PyRx software. The results will be obtained through affinity values and ligand binding to the target protein or receptor (Sari et al., 2020).

Method validation

Validation is a comparison stage between natural and artificial ligands that will be tested against receptors experimentally with the same ligand position results from the docking process. The basis for determining validation is the value of Root Mean Square Deviation (RMSD). The method is valid if the RMSD value is <2Å (Gaspersz & Sohilait, 2019).

Data Analysis

The affinity results obtained from the six anthocyanin-derived compounds were then compared to each natural ligand, Angiotensin bonds in two-dimensional and three-dimensional forms using the Discorverystudio 2016 Client program.

RESULTS AND DISCUSSION

Analysis of Chemical Physics Properties of Ligand

The structure of anthocyanin-derived compounds and controls can be seen using the ChemDraw application in two dimensions and continued analysis on the "pkCSM" web. The compound structure can be seen in Table 2. The results of the analysis of physicochemical properties of compounds based on the structure to see the number of H donors (NH and OH) and H acceptors (N and O), molecular weight, and log P (Table 2). According to Lipinski's rule, the requirement of an H donor is not more than five, and an H acceptor is not more than 10 to have good permeability. Based on the structural analysis that has been done, it is known that the higher the hydrogen bonding capacity, the higher the energy required for the absorption process to occur. According to Lipinski's rule, the molecular weight of compounds that can penetrate biological membranes is no more than 500g/mol. Molecular weights over 500g/mol cannot diffuse through cell membranes (Hardjono, 2016).

Table 3. Docking results and interaction types

Reseptor	Compound	Afinitas (*ΔG*)	Hydrogen bond residues (± 2 Å)	Residue Other bonds (± 2Å)
ACE	Delphinidin	-7.7	ASP415(2.63 Å)	GLU162(2.99 Å)
	Petunidin	-7.8	ASP415(2.38 Å),	GLN281(2.51Å)
	Malvidin	-7.7	ASP377 (2.54Å), TYR520(2.83 Å), ASP415 (2.73 & 2.90 Å),	-
	Cyanidin	-7.7	ASP415(2.30 Å), ASP377(2.14 Å), HIS383 (2.82&4.81 Å)	ASP377(2.94 Å)
	Peonidin	-7.8	GLU162(2.42 Å), ASP415(2.64 Å),	
	Pelargonidin	-7.7	GLU162(2.59 Å), ASP415(2.54 Å), TYR520(2.12 Å),	
	Lisinopril (Kontrol/ Comparator	-8.4	GLN281 (2.19&2.76 Å), HIS353 (2.13Å), HIS513 (2.75Å), ALA353 (2.59&2.78 Å), TYR523 (2.13Å), HIS387(2.43 Å),	GLU384(2.48 Å)
	Captropil (Kontrol/ Comparator)	-5.7	HIS353 (2.15Å), HIS513(2.12 Å), TYR523 (2.54&2.63)	
AT1	Delphinidin	-7.7	ARG167(2.55 Å), TYR92(2.88Å), THR88(2.56 Å), TRP84 (2.64&2.92 Å)	-
	Petunidin	-7.7	SER109(2.88 Å) SER105(3.35 Å), THR88(2.57 Å), TRP84(2.69 Å)	-
	Malvidin	-7.8	TYR92(2.96 Å), SER105(3.38 Å), ARG167(2.54 Å), TRP84(2.63 Å)	-
	Cyanidin	-7.7	THR88(2.53 Å), TRP84(2.92 Å)	-
	Peonidin	-7.8	SER105(3.44 Å), THR88(2.53 Å)	-
	Pelargonidin	-7.6	TYR35(2.50 Å),	-
	Losartan (Kontrol/ Comparator	-8.6	ARG167(2.73Å), TYR35(2.84 Å)	-
	Olmesartan (Kontrol/ Comparator	-9.1	ARG167 (2.49,2.62&2.64 Å)	-

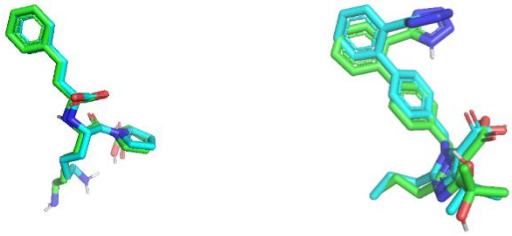
Note:
● = Amino acid residue of hydrogen bond is the same as drug control

The negative log P value is unfavorable as the molecules cannot pass through the lipid bilayer membrane. The log P value describes the ability of the compound to dissolve in biological membrane fluids. The greater the log P value, the more hydrophobic the molecule (Nindita & Sanjaya, 2014). Highly hydrophobic molecules tend to have higher toxicity because they are retained longer in

the lipid bilayer and are more widely distributed in the body, resulting in reduced binding selectivity to target proteins (Bogdanov et al., 2008).

Method validation results

The results of re-docking of protein ligands show the Root Mean Square Deviation (RMSD) value on the natural ligand lisinopril of 1.347Å and Olmesartan ligand of 1.618Å. The validation results of the original ligand position and the copy ligand position can be seen in Figure 2.



Lisinopril (ACE) Olmesartan (AT1)

Figure 2. Overlay of original ligand position (green) and Copy ligand (blue)

indicates that the smaller the error of the calculation results, the more accurate the calculation, and the protein is said to be valid (Ferwadi, 2017).

Data Analysis

The docking process was carried out using the PyRx application based on the Vina program, with the parameters of the docking results seen are the value of Gibbs free energy (ΔG) and Root Mean Square Deviation (RMSD). The small Gibbs free energy value indicates that the conformation formed is stable (Pantsar & Poso, 2018; Wolf, 2009). Receptors and ligands that have been optimized are entered into the PyRxprogram. Then a grid box is set up as a place or coordinate where the ligand will interact with the target receptor visualized as a cube. The grid box setting is done by adjusting the coordinates of the active side of the natural ligand of each receptor (Harini et al., 2021).

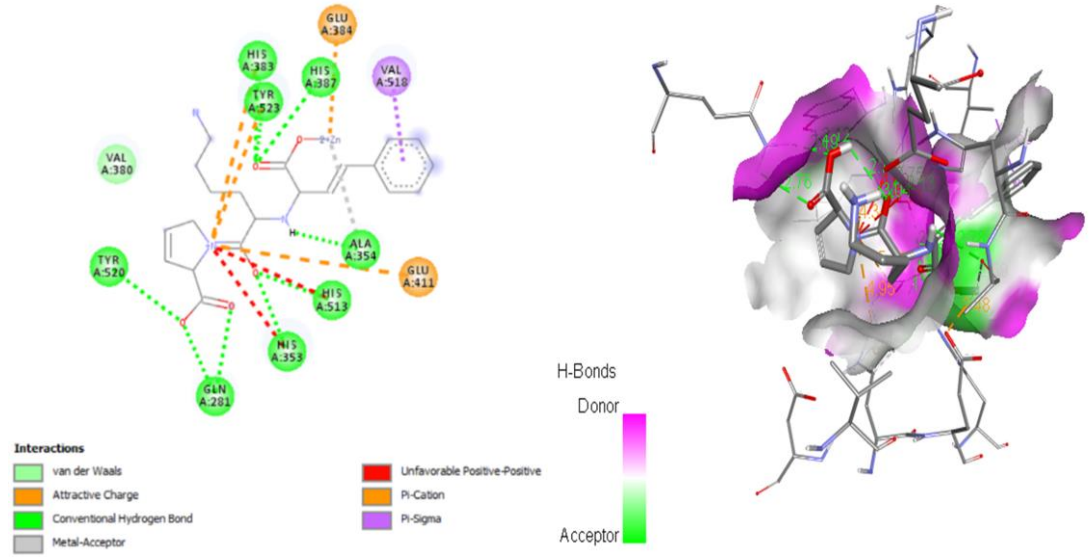
The six anthocyanin-derived compounds were then paired with Angiotensin Converting Enzyme (ACE) protein at coordinates, center x = 41.27, center y = 32.40, center z = 8.06, with grid box size x = 9.85, size y=15.34 size z=16.01. Angiotensin

Table 4 Number of residue bonds to target protein

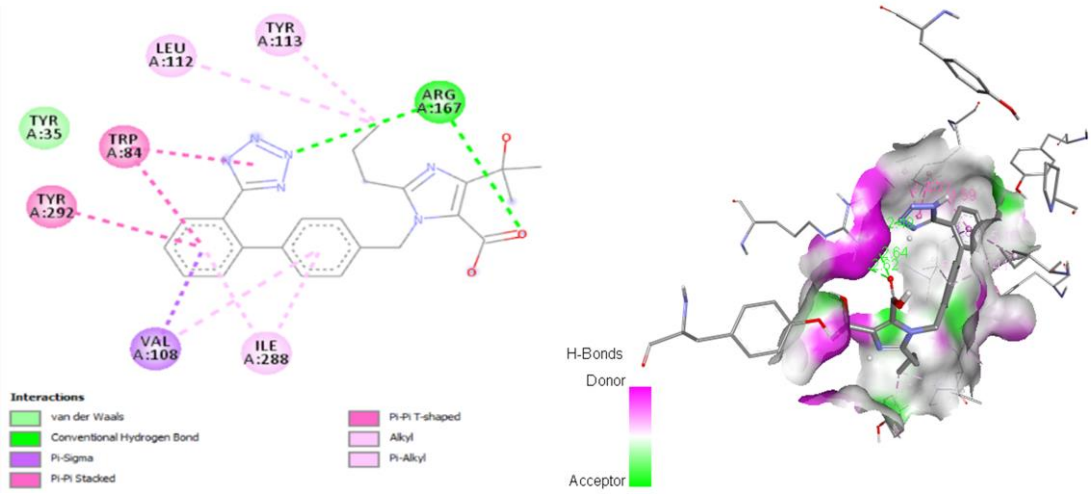
Type of Protein	Test Compound	Residual Bond Amount (± 2 Å)	Same number of bonds as the control compound (± 2 Å)	
			Hydrogen Bonding	Beyond Hydrogen Bonding
ACE	Delphinidin	2	0	1
	Petunidin	2	0	1
	Malvidin	4	1	0
	Cyanidin	4	0	0
	Peonidin	3	0	1
	Pelargonidin	2	0	0
AT1	Delphinidin	5	1	0
	Petunidin	4	0	0
	Malvidin	4	1	0
	Cyanidin	2	0	0
	Peonidin	2	0	0
	Pelargonidin	1	1	0

Root Mean Square Deviation (RMSD) validation results show that the atomic position on the ligand from the re-docking result (ligand copy) is not much different from the position on the crystallography result ligand (original ligand). The magnitude of the RMSD value and the overlap of the crystallographic ligand and the re-docking ligand shows the RMSD value < 2 Å. This number

Receptor (AT1) is paired at coordinates, center x= -40.31, center y= 63.99 center z= 27.60 with grid box size x=15.60, size y=15.81 size z=13.96. The affinity values of all test compounds and control compounds show the bond that occurs to the receptor, where in theory, the lower the affinity energy, the compound will quickly bind to the (Ding et al., 2016).



(A)



(B)

Figure 2. (A) Interaction native ligand lisinopril with angiotensin converting enzyme ACE, (B) Interaction native ligand olmesartan with angiotensin receptor (AT1)

The lowest and best affinity values were shown by the positive control lisinopril of -8.4 kcal/mol and the test ligand Malvidin of -7.7 kcal/mol, having three types of hydrogen bonds at a distance of ± 2 Å (ASP377, TYR520, ASP415) and have 1 type of bond in common with the control drug lisinopril (TYR520) at the Angiotensin Converting Enzyme (ACE) receptor While for Angiotensin Receptor (AT1) the lowest affinity value is shown in the control compound Olmesartan at -9.1 kcal/mol and Malvidin test ligand of -7.8 kcal/mol have four types of hydrogen bonds ± 2 Å distance (TYR92, SER105, ARG167, TRP84) and have 1 type of bond that is the same as the control drug lisinopril (TYR520).

Some of the most commonly encountered types of bonds, especially in computational chemistry research, are Van Der Waals Interactions, where the interaction mechanism is in the form of attraction between molecules of hydrophobic group atoms such as aromatic ring and alkyl groups (Adeniji et al., 2020; Ferrighi et al., 2012; Matsusaki et al., 2012). Another bond commonly encountered in

computational chemistry or molecular docking is the hydrogen bond, which consists of N, I, and F atoms that bind to the H atom (Widiastuti, 2019). Then there is the pi(π) bond which consists of a covalent chemical bond of two single-electron atomic orbital lobes overlapping with two other atomic orbital lobes, which are also single-electrons.

Table 3 showed the same amino acid interaction as the control drug in Angiotensin Converting Enzyme (ACE), Glutamine (GLN), Histidine (HIS), Tyrosine (TYR) in H-Donor, and Valine (VAL), Alaninal (ALA) in alkyl. While in Angiotensin Receptor (AT1), Arginine (ARG) in H-Donor, Isoleucine (ILE), Leucine (LEU) in alkyl, Tyrosine (TYR), Tryptophan (TRP) in Pi-Orbitals.

Table 4 shows the number of amino acid residue bonds that are the same as the drug control used and enter the range on the active side of the target amino acid so that it can be presumed to have the exact mechanism of action and bonding as the drug control compound used (Arwansyah et al., 2014). The bond distance can affect the pharmacological effect of a drug or compound. If the bond distance is not appropriate, then the effect provided by the drug can be reduced (Erdmann & Schwarz, 2007). Bond distance is influenced by the type of chemical structure between the protein and ligand. Bonding of amino acid residues from hydrogen bonds ranges from 2-3Å while amino acid residues from other bonds (carbon-hydrogen bonds, sigma pi(π) bonds) ranges from 2-5Å.

Table 3 shows the number and interactions between ligand-receptors entering the same range of active residues against the positive control. Petunidin has the highest value of the six anthocyanin-derived compounds, with nine amino acid residue bonds entering the active side of the Angiotensin Converting enzyme (ACE). In contrast, Malvidin has the highest value, with seven amino acid residue bonds entering the active side of the Angiotensin Receptor (AT1). Anthocyanin-derived compounds, namely, Delphinidin, Petunidin, Malvidin, Cyanidin, Peonidin, and Pelargonidin, can fulfill pharmacodynamic parameters and are predicted to be anti-hypertensive candidates through the Renin-Angiotensin-Aldosterone System (RAAS).

Based on in silico testing that has been done, the five anthocyanin compound derivatives interact better with Angiotensin Converting Enzym (ACE) proteins. These results are supported by several reviews and research on Rosella flowers (Hibiscus sabdariffa) which contain several anthocyanin derivatives that can work actively in inhibiting Angiotensin Converting Enzyme (ACE) activity (Kusumastuti, 2014; Ojeda et al., 2010; Wahabi et al., 2010).

CONCLUSION

Based on the results of the research that has been done, it can be concluded that anthocyanin-derived compounds, namely Delphinidin, Petunidin, Malvidin, Cyanidin, Peonidin, and Pelargonidin, have potential as hypertension drugs through the Renin-Angiotensin-Aldosterone (RAAS) pathway based on molecular docking calculations. The affinity value of each, against Angiotensin Converting Enzym (ACE) are -7.7; -7.8; -7.7; -7.7; -7.8 and -7.7. The best affinity value in anthocyanin-derived compounds are shown in the Malvidin test compound which has three types of hydrogen bonds at a distance of ± 2 Å (ASP377, TYR520, ASP415) and has 1 type of bond that is the same as the control drug lisinopril (TYR520). At the same time, the affinity value to Angiotensin Receptor (AT1R) are -7.7; -7.7; -7.8; -7.7; -7.8, and -7.6, respectively. The best affinity value is shown in the Malvidin test ligand compound of -7.8 kcal/mol having four types of hydrogen bonds ± 2 Å distance (TYR92, SER105, ARG167, TRP84) and has 1 type of bond that is the same as the control drug lisinopril (TYR520). There are hydrogen, pi (π), and Van Der Waals bond interactions of Delphinidin, Petunidin, Malvidin, Cyanidin, Peonidin, and Pelargonidin compounds against Angiotensin Converting Enzyme (ACE) and Angiotensin Receptor (AT1) which have similar bonds in each drug control, with an interaction distance of ± 2 Å, where this distance can already bind well to the protein target so that it can provide a good pharmacological effect on reducing hypertension in the body.

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