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In Silico Study of Compound Extract in Soursop Plant (*Annona muricata*) as ACE Inhibitor in Hypertension Disease

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Hypertension is one of the risk factors for cardiovascular disease that has become a serious global health problem. In terms of searching for active compounds that have the potential as antihypertensives, phytochemical studies on natural ingredients are continuously being carried out. One of them is soursop which is known to be able to lower blood pressure in people with hypertension. Alkaloids contained in soursop leaves can work as Angiotensin Converting Enzyme Inhibitor hat reduce ADH hormone secretion. This research was conducted to find out the antihypertensive potential of natural compounds in soursop leaves that interact with ACEI. The research was carried out in two steps, namely molecular anchoring and pharmacophore modeling. Based on the research that has been done, Anomuricine is the compound with the most potential interaction with ACEI with a Gibbs free energy of -9.13 kcal/mol and an inhibition constant of 202.83 uM. In addition, the Anomuricine compound has a lower binding energy than the standard ligand binding energy. Thus, it can be said that Anomuricine from soursop leaves (Annona muricata) has the potential as a lead compound.

Keywords: ACEI | Hypertension | Molecular Docking | Natural Compounds | Pharmacophore Modeling

Hypertension is defined as a condition in which there is a persistent increase in blood pressure. Hypertension is a serious health problem because it is a risk factor for cardiovascular disease (1). Based on the 2018 Basic Health Research conducted on Indonesians aged 18 years and over, it is known that the incidence of hypertension has increased to 34.1% compared to 2013 where the prevalence of hypertension is 25.8% (2). The prevalence of hypertension is expected to increase every year and has cost a lot of health care. Thus, hypertension is a serious global health problem (3).

The current management of hypertension conditions shows a fairly complicated polemic. Handling of hypertensive patients needs to be done by looking at the severity of the patient. Even in some conditions, patients with low hypertension are even more at risk for experiencing side effects from the use of drugs, such as hypotension, syncope, and acute kidney injury (4). Therefore, the search for antihypertensive drug candidates with better safety profiles is necessary.

In recent decades, exploration of potential ingredients in natural ingredients has become the main alternative to find relatively safer drug candidates. Soursop (*Annona muricata* Linn) is known to be able to reduce blood pressure in people with hypertension because it contains antioxidants that can ward off free radicals which can flex, dilate blood vessels, and lower blood pressure (5). The alkaloids contained can act as ACE inhibitors because of their ability to reduce the secretion of antidiuretic hormone (ADH) which increases urine secretion, NaCl secretion, and decreasing blood pressure (6,7). In this study, a molecular docking simulation was conducted on the content of alkaloid compounds in soursop towards Angiotensin Converting Enzyme-I (ACE-I) as one of the essential targets of antihypertensive agents. The simulation process was carried out in silico using AutoDock software. In addition to finding good drug candidates by activity, in this study, screening was also carried out to determine the pharmacokinetic properties and toxicity profiles of compounds using SwissADME, a web-based software. The final goal of this research is to screen the most promising compound as candidate of an anti-hypertensive agent contained in the soursop plant.

Method

Equipment

The hardware used was a personal computer with specifications Intel® Core™ i3-8145U/4Gb RAM/512G PCIe/Windows 10 Home, and AMD Ryzen 7 5700U with Radeon Graphics (16 CPUs), 1.8GHz and 8 Gigabyte RAM. The in silico assay used software which included: ChemOffice 2010 and ChemDraw Ultra 12.0 programs (PerkinElmer Inc.), AutoDock 4.2.6 and AutoDockTools 1.5.6 (The Scripps Research Institute) programs, BIOVIA Discovery Studio 2017 program, Ligandscout program, and SwissADME online program.

Materials

The materials used included the three-dimensional structure of the ACEI enzyme from the Protein Data Bank (PDB) (www.rcsb.org) with the code 4CA5. Three-dimensional structure of natural ligand and test compounds were downloaded from Pubchem (https://pubchem.ncbi.nlm. nih.gov/) which was prepared using ChemOffice 2010 software.

Detail Procedure

2D modeling was performed using Chemdraw MarvinSketch. The 3D modeling was then carried out using the software Chem3Dand then the geometry optimization was performed using LigandScout. Determination of the physicochemical properties of each compound was carried out online at the SwissADME database http://www.swissadme.ch/index.php, then it was analyzed based on the Lipinski Rule of Five. Files were downloaded in the form of "*.mol*".

ADMET predictions were performed on the https://preadmet/bmdrc.kr/ page and the structure of the test compound was drawn on the column provided, then press "submit" until information was obtained. Toxicity information was obtained by pressing the "Toxicity" task by clicking on the upper right corner

and drawing the compound to be tested in the image column then pressing "submit". The information obtained was downloaded in PDF form. These same steps are performed on each ligand.

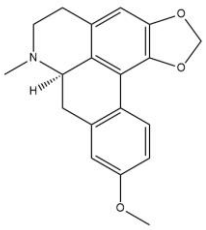
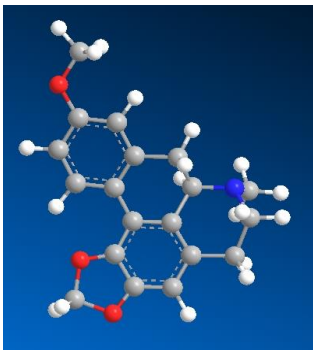
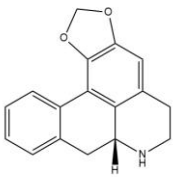
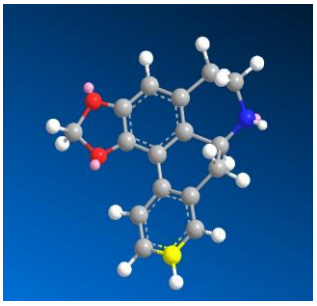
Molecular docking simulation was carried out by constructing a ligand structure using chemdraw and converting it to ".pdb" format using chem3d. The ligands were opened using autodock and then computed gasteiger charges were added to the ligands. Hydrogen atom are also incorporated into the ligands and merge the non-polar bonds of the ligands. Then, the ligands were inputted and added by the torsion tree. Ligands were stored in ".pdbqt" format. The receptor was prepared by downloading the ACEI receptor from www.rcsb.org with the code 4CA5. The receptor was opened with the Biovia Discovery Studio app. The water molecules and the bound ligand molecules were removed from the receptor. ACEI receptors (4CA5) were stored in the form of ".pdb" format and opened using autodock. Then the Kolman charge and polar bonds were added to the receptor and the Grid parameter was arranged by determining the coordinates and area of the active pocket of the ACEI receptor using the grid box feature of the autodock. Results were saved in "dock.gpf" format. Docking simulation was carried out with ligands and receptors using Genetic Algorithm parameters with 100 runs. Furthermore, docking was done using the command prompt (cmd) and analyzed by determining the best confirmation by looking at the Gibbs Energy, KI, and RMSD of each ligand and receptor docking results. After that, the interaction that occurs between the ligand and the receptor was determined.

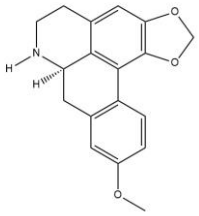
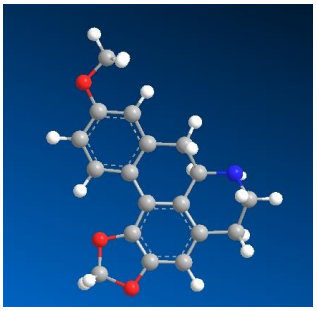
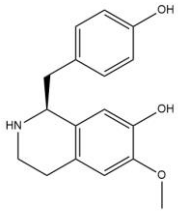
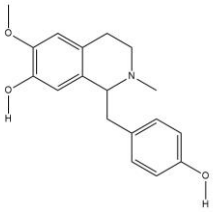
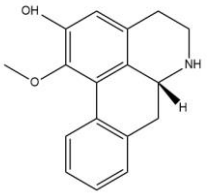
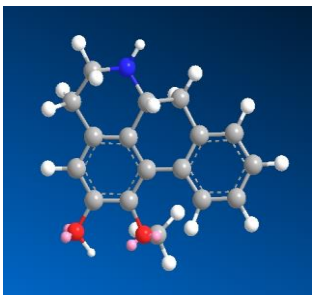
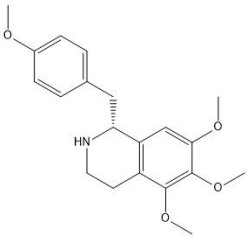
The pharmacophore modeling was carried out by downloading the ACEI receptor from the Protein Data Bank Website www.rcsb.org with the code 4CA5. Then the human estrogen receptor alpha was found with PDB ID: 3ERT; (GZ PDB Format for MacOS). The file was opened with LigandScout software and a pharmacophore was created and identified positively ionized, hydrophobic, hydrogen bond donor and hydrogen bond acceptor on the pharmacophore. The existing pharmacophore results were then saved in 2D and 3D in jpg/png format with the desired resolution. The properties of the available ligands could be viewed by clicking on the Ligand details tab.

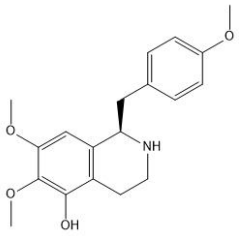
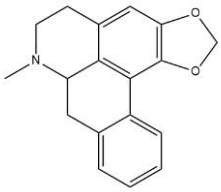
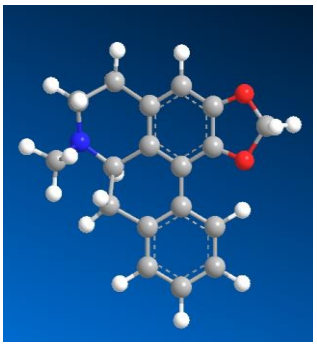
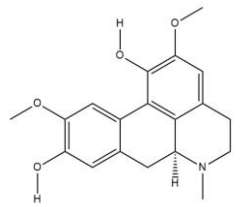
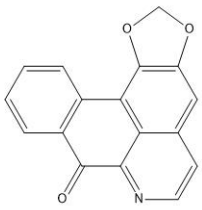
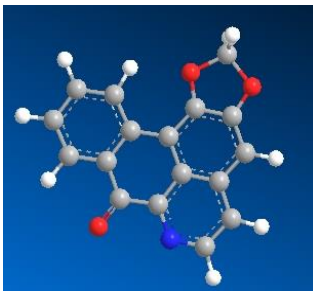
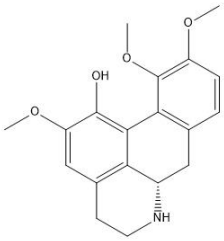
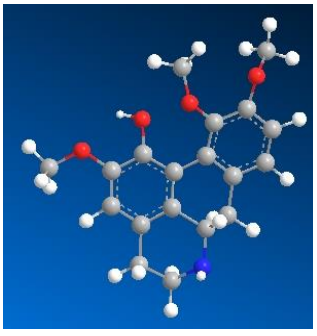
Result

Prediction of the physicochemical properties of the compounds contained in soursop was carried out based on the Lipinski Rule of Five. The results of this analysis showed that all the test compounds met the requirements for acceptance of Lipinski's rules, thus, they could be made into oral dosage forms. The prediction results and the structure of the test compounds were presented in Table 1. In Table 2. ADMET predictions were performed using ADMETlab 2.0 and pkCSM showed that all compounds in plants had good absorption because they were in the range of 70-100% in the results of Human Intestinal Absorption (HIA) and strongly bound based on Plasma Protein Binding (PPB) results. While the distribution to the brain barrier was low because the Blood Brain Barrier (BBB) value was less than 0.1. The drug permeability was low because the results of Human Colon Adenocarcinoma (Caco-2) cells were below 4. The results of the toxicity show that there are only three compounds that are neither carcinogenic nor mutagenic, they were isoboldine, anomimuricine, and (S)-norcorydine compounds.

Table 1. Prediction of Physicochemical Properties

No.	*Compound*	*Description*	*2D Structure*	*3D Structure*
1.	Isolaureline	Formula = $C_{17}H_{15}NO_2$ Molecular Weight = 265.31 g/mol Log P = 3.12 Hydrogen Bonds: - Donor = 0 - Acceptor = 4		
2.	Anonaine	Formula = $C_{19}H_{19}NO_3$ Molecular Weight = 309.36 g/mol Log P = 2.89 Hydrogen Bonds: - Donor = 1 - Acceptor = 3		

3.	Xylopine	Formula = $C_{18}H_{17}NO_3$ Molecular Weight = 295.33 g/mol Log P = 2.88 Hydrogen Bonds: - Donor = 1 - Acceptor = 4		
4.	Coclaurine	Formula = $C_{17}H_{19}NO_3$ Molecular Weight = 285.34 g/mol Log P = 2.36 Hydrogen Bonds: - Donor = 3 - Acceptor = 4		
5.	N-Methylcoclaurine	Formula = $C_{18}H_{21}NO_3$ Molecular Weight = 299.36 g/mol Log P = 2.59 Hydrogen Bonds: - Donor = 2 - Acceptor = 4		
6.	Asimilobine	Formula = $C_{17}H_{17}NO$ Molecular Weight = 267.32 g/mol Log P = 2.65 Hydrogen Bonds: - Donor = 2 - Acceptor = 3		
7.	Anomurine	Formula = $C_{20}H_{25}NO_4$ Molecular Weight = 343.42 g/mol Log P = 3.08 Hydrogen Bonds: - Donor = 1 - Acceptor = 5		

8.	Anomuricine	Formula = $C_{19}H_{23}NO_4$ Molecular Weight = 329.39 g/mol Log P = 2.65 Hydrogen Bonds: - Donor = 2 - Acceptor = 5		
9.	Remerine	Formula = $C_{18}H_{17}NO_2$ Molecular Weight = 279.33 g/mol Log P = 3.13 Hydrogen Bonds: - Donor = 0 - Acceptor = 3		
10.	Isoboldine	Formula = $C_{19}H_{21}NO_4$ Molecular Weight = 327.37 g/mol Log P = 2.45 Hydrogen Bonds: - Donor = 2 - Acceptor = 5		
11.	Liriodenine	Formula = $C_{17}H_9NO_3$ Molecular Weight = 275.26 g/mol Log P = 2.88 Hydrogen Bonds: - Donor = 0 - Acceptor = 4		
12.	(S)-norcorydine	Formula = $C_{19}H_{21}NO_4$ Molecular Weight = 327.37 g/mol Log P = 2.61 Hydrogen Bonds: - Donor = 2 - Acceptor = 5		

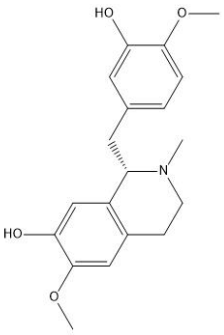
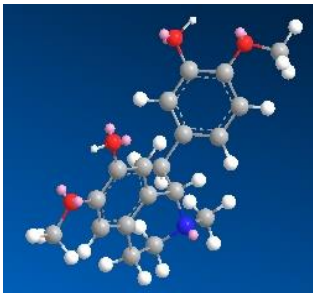
13.	Reticuline	Formula = $C_{19}H_{23}NO_4$ Molecular Weight = 329.39 g/mol Log P = 2.64 Hydrogen Bonds: - Donor = 2 - Acceptor = 5		

Table 2. ADME Toxicity Prediction

No.	*Compound*	*Absorption*		*Distribution*		*Toxicity*	
		HIA (%)	*Caco-2 (nm/sec)*	*PPB (%)*	*BBB*	*Mutagenicity*	*Carcinogenicity*
1	Anonaine	96.245	-4.95	90.02%	0.245	Yes	Yes (0.715)
2	Isolaureline	93.934	-4.822	94.26%	0.591	Yes	Yes (0.79)
3	Xylopine	98.449	-5.081	88.85%	0.013	Yes	Yes (0.341)
4	Coclaurine	88.708	-5.042	50.67%	0.028	Yes	No (0.108)
5	N-Methylcoclaurine	88.498	-4.698	76.43%	0.136	Yes	No (0.095)
6	Asimilobine	93.426	-5.099	73.71%	0.169	Yes	No (0.045)
7	Remerine	92.258	-4.7	94.27%	0.652	No	Yes (0.814)
8	Isoboldine	90.749	-4.86	84.74%	-0.17	No	No (0.053)
9	Anomuricine	94.054	1.25	67.583%	-0.074	No	No (0.036)
10	Anomurine	96.976	-4.948	72.465%	0.249	Yes	No (0.032)
11	Liriodenine	99.719	1.294	97.984%	0.249	Yes	Yes (0.946)
12	(S)-norcorydine	92.653	1.234	82.882%	-0.234	No	No (0.044)
13	Reticuline	90.796	0.904	68.023%	-0.049	Yes	Yes (0.102)

The results of the molecular docking simulations in Table 3 and Table 4 indicated that anomuricine has a lower bond energy than the standard ligand, which was -9.13 kcal/mol. The validation of the molecular docking was done by re-docking between the receptor and natural ligands. The receptor used has the PDB code 4CA5 which was an angiotensin-converting enzyme in humans that formed a complex with Fi Tripeptide Phosphinate. Crystal structure of the two human ACE catalytic domains (N- and C-) in complex with FI, the S enantiomer of the phosphinic ACE/ECE-1 (endothelin converting enzyme) FII double inhibitor, have a resolution of 1.91 and 1.85 respectively (8). The best results obtained were the RMSD value of 1.51, with an RMSD value of less than 2 as much as 12%, and an RMSD value of less than 3 which was 96%. It was fulfilled the best criterion of the method validation of molecular docking that should not less than 70% of result which presented an RMSD value of or less than 2 (9). For the pharmacophore in Figure 1, the following results were obtained: 4 hydrogen bonds: ARG 522, ALA 354, HIS 513, HIS 383; 10 carbon-hydrogen bonds: GLU 143' SER 516, PHE 512, SER 355, GLY 404, PRO 407, PHE 391, ASP 415, PHE 527, PHE 457.

Table 3. Molecular Docking Results of Natural Ligand

No	*Compound*	*Cluster*	*Binding energy (kkal/mol)*	*Ki (uM)*	*Amino acid's interactions*		
					Hydrogen Bond	*van der waals Interaction*	*Others*
1	N-{(2s)-3-[(S)-[(1r)-1-{[(Benzyloxy)carbonyl]amino}-2-Phenylethyl](Hydroxy)phosphoryl]-2-[(3-Phenyl-1,2-Oxazol-5-Yl)methyl]propanoyl}-L-Tyrosine	2	-8.95	275.30 nM	ARG 522 ALA 354 HIS 513 HIS 383	VAL 518 HIS 410 HIS 387 ALA 356 TYR 523 VAL 380	Carbon Hydrogen Bond GLU 143 SER 516 PHE 512 SER 355 GLY 404 PRO 407 PHE 391 ASP 415 PHE 527 PHE 457 Attractive Charge GLU 384 GLU 411 Unfavorable Positive-Positive HIS 353

Table 4. Molecular Docking Results of Test Compounds

No	*Compound*	*Cluster*	*Binding energy (kkal/mol)*	*Ki (uM)*	*Amino acid's interactions*		
					Hydrogen Bond	*van der waals Interactions*	*Others*
1.	Anonaine	1	-8.30	819.40 nM	GLU 386	-	Pi-Anion ASP 415 Pi-Alkyl VAL 380 Pi-Pi Stacked TYR 383, HIS 383
2.	Isolaureline	1	-8.62	477.54 nM	GLN 281, LYS 511	-	Pi-Pi T-Shape HIS 383 Alkyl HIS 353
3.	Xylopine	1	-8.47	614.00 nM	LYS 511, GLN 281	-	Pi-Pi T-Shape HIS 383
4.	Coclaurine	1	-8.28	858.13 nM	HIS 383, ASP 415	-	Pi-Pi Stacked TYR 523 Pi-Alkyl VAL 380

v5.	N-Methylcoclaurine	2	-7.11	6.18 uM	HIS 353, GLU 384	-	Pi-Anion GLU 411 Pi-Pi Stacked TYR 523, HIS 387 Alkyl VAL 518
6.	Asimilobine	1	-8.35	752.99 nM	GLU A: 384	-	Pi-Alkyl ALA 356, VAL 518 Pi-Anion GLU 411
7.	Remerine	1	-8.26	886.24 nM	HIS 513 GLN 281 LYS 511	-	Pi-cation HIS 353 Alkyl ALA 354
8.	Isoboldine	3	-7.34	4.19 uM	HIS 363	HIS 387 TYR 523	Carbon Bond ALA 356 Hydrogen Pi-Anion GLU 411
9.	Anomuricine	1	-9.13	202.83 nM	LYS 511 GLN 281 GLU 384 ALA 354 ALA 356	HIS 387	Pi-cation HIS 353
10.	Anomurine	2	-8.69	425.35 nM	GLU 384	HIS 387 PHE 457	Carbon Bond ALA 356 Hydrogen ASP 415
11.	Liriodenine	1	-6.86	9.40 uM	GLU 384	HIS 383 VAL 380 ALA 354 TYR 523	Pi-Anion ASP 415
12.	(S)-norcorydine	1	-8.05	1.25 uM	HIS 513 HIS 353 HIS 354	TYR 523	Carbon Bond ALA 356 Hydrogen Pi-Anion GLU 411
13.	Reticuline	2	-7.69	2.32 uM	HIS 353 TYR 523 HIS 513 TYR 520 LYS 511	HIS 383	Carbon Bond ASP 415 Hydrogen

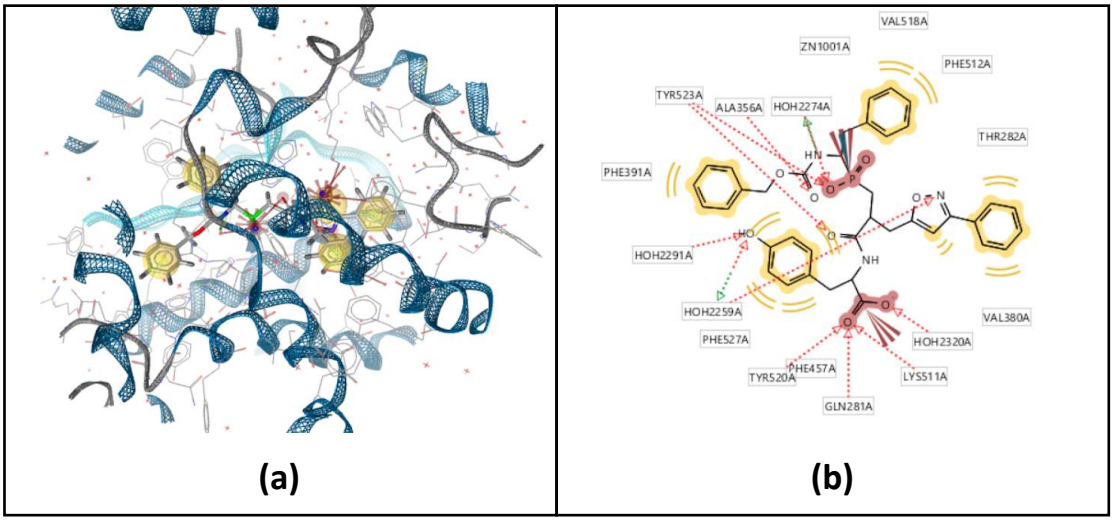


Figure 1. Result of pharmacophore modelling: (a) ligand-active pocket simulation and (b) ligand's pharmacophore-active site interaction

Discussion

The in silico study of soursop as an ACE inhibitor began with an analysis of the test compounds contained in soursop based on the Lipinski Rule of Five to determine the physicochemical properties of the test compounds related to their permeability to the lipid bilayer in achieving targets in the body. Lipinski's requirements consist of several parameters, which were molecular weight (BM) not more than 500 Da, not having a partition coefficient value (logP) more than 5, the number of donor bonds and hydrogen bonds must be less than 5, and bond acceptors must be less than 10 (10).

BM shows the ability of compounds to penetrate the membrane, the greater or > 500 Da, the more difficult it is to penetrate the membrane. The log P value indicates lipophilicity, where the greater than 5, the more lipophilic the compound or very strongly bound to the membrane which makes it difficult to identify the target enzyme and is toxic. However, smaller value also makes it more difficult for the compound to penetrate the lipid bilayer membrane. The hydrogen bond donors and acceptors show the amount of hydrogen bonding capacity, where the higher the hydrogen bond, the higher the energy required for the absorption process (11).

Prediction of ADMET which consists of Absorption, Distribution, Metabolism, Excretion and Toxicity. The PPB results are strongly bound if the yield is more than 50% and weakly bound if it is below 50%. The better the bond, the better the distribution of the drug in the blood. A compound is said to have poor absorption if the result of HIA is 0-20%, moderate absorption at 20-70% and good absorption at 70-100%. While BBB which is the ability of blood vessels to vascularize the central nervous system. The value of CaCo-2 is a parameter that is seen to determine the permeability at the time of absorption where if the result is below 4 then the permeability is low, while 4-70 is moderate and above 70 is high. Form the experiment, all of test compounds has good pharmacokinetic profiles, which were good absorption and strongly bonded to protein. However, only three compounds which has good prospective by their good toxicity profiles, carcinogenicity and mutagenicity. They were Isoboldine, Anomuricine, and (S)-norcorydine. Interestingly, Isoboldine, an aporphine alkaloid, was discovered to has various activity, such as anti-inflammatory, anti-oxidant, and anti-cancer agent (12,13). (S)-norcorydine and Anomuricine, isoquinoline alkaloids, also has anti-cancer and anti-infective activity (14,15).

Molecular docking validation was aimed to see how accurate the prediction data results from data processing applications were with tests carried out directly in the laboratory. Accuracy in validation was assessed by looking at the RMSD value. The RMSD (root mean square deviation) value determines how well the docking combination forms the ligand at the protein site. RMSD was used to evaluate how different the docking orientations obtained from the corresponding co-crystallization poses of the same ligand molecule. A good RMSD value is less than or equal to 2, an RMSD value of 2-3 is still acceptable. The smaller the RMSD value, the greater the docking accuracy (16–18). The results of the validation with the PDB receptor 4AC5 meet the requirements of the RMSD value for validation.

Valid molecular docking simulation was then carried out to compare the binding energies of the standard ligand and the test compound to the ACEI (4CA5) receptor. The best bond energies are those with the lowest values. From the bond energies of Isoboldine, Anomuricine, and (S)-norcorydine with standard ligands, Anomuricine has a lower bond energy than standard ligands of -9.13 kcal/mol. Therefore, Anomuricine has the most potential as a lead compound. This compound was long found and isolated in 1980th by Leboeuf et al (19,20).

Furthermore, a pharmacophore model study was conducted. Pharmacophores are part of the structure of drug compounds that are needed to ensure optimal interactions of compounds with certain biological targets and trigger or block their biological responses. The pharmacophore model was obtained using the software, LigandScout, where the 3D structure of the ligand-protein complex obtained from the Protein Data Bank was used. As depicted in Figure 1, we could imply that the four benzene's rings and four hydrogen acceptors (oxygen atom) were the main part of the structure which have a main role in interacting with the active site of the ACE-I.

Conclusion

Based on the study that have been carried out, it was known that natural compounds such as Isoboldine, Anomuricine, and (S)-norcorydine, have antihypertensive potential in overcoming hypertension by binding to ACEI receptors (4CA5). The compound with the highest potency was Anomuricine with a Gibbs free energy of -9.13 kcal/mol and an inhibition constant of 202.83 uM. In addition, the Anomuricine compound has a better pharmacokinetic of absorption profile thus it was potential to be formulated in oral preparation and has low toxicity (non-mutagenic and non-

carcinogenic) profiles. Thus it can be said that Anomuricine from soursop leaves (*Annona muricata*) has the potential as a lead compound.

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