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Active Compounds of Sembung Leaves (*Blumea balsamifera* DC) in Silico Screening as Antihypertensives

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ABSTRACT

Hypertension is a structural or functional change in the arteries or the organs it supplies caused by increased blood pressure. Angiotensin-converting enzyme (ACE) can increase blood pressure by converting inactive angiotensin I to active (angiotensin II). Captopril is a hypertension drug that can inhibit ACE activity. Sembung leaf (*Blumea balsamifera* DC) is a plant that can potentially have antihypertensive activity. This study aims to identify the interaction of active compounds in sembung leaves against ACE as antihypertensive drug candidates through an in silico test based on pharmacodynamic and pharmacokinetic parameters using two docking software, Autodock Vina and PyRx. The results showed that Luteolin was the best test ligand besides having lower ΔG and Ki than control ligands and higher Kd than control ligands. Luteolin was identified to interact with Zn and hydrogen bond interactions at the active site and met the criteria in Lipinski analysis, ADME, and toxicity, so this compound is relatively safe to be used as a drug candidate for the treatment of hypertension.

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1. Introduction

Hypertension is a widespread health issue, especially in Indonesia. The World Health Organization (WHO) estimates that 22% of the world population has hypertension, with Southeast Asia ranking third at 25%. In 2018, the prevalence rate of hypertension in the Indonesian population over 18 years old was 34.11%, an increase from 25.8% in 2013. WHO data from 2015 shows that 1.13 billion people worldwide suffer from hypertension, meaning one in three individuals globally. It is projected that by 2025, 1.5 billion people will be affected by hypertension, and approximately 10.44 million deaths occur each year due to hypertension and its complications [1].

Sembung leaf (Blumea balsamifera DC) is a medicinal plant known to contain flavonoids, particularly Quercetin, which has ACE inhibitor properties. Quercetin in sembung leaves can reduce oxidative stress, inhibit angiotensin-converting enzymes, and promote blood vessel relaxation, as stated by Ruhardi and Handoyo Sahumena [2]. Sutjiatmo et al. [3] found that different doses of ethanol extract from sembung leaves (*Blumea balsamifera*) showed potential as antihypertensive agents in animal test models induced by epinephrine. Afrianti et al. [4] demonstrated that the ethanol extract of sembung leaves could effectively reduce blood pressure in white male rats, with the most significant effect observed at a dose of 500 mg/kg BW. Trivadila et al. [5] discovered that the polar layer of tempuyung leaf extract exhibited antihypertensive properties comparable to the synthetic drug captopril when tested using the in vitro method.

In an in silico study conducted by Utari et al. [6], it was found that Quercetin, a compound present in sembung leaves, exhibited activity against the angiotensin-converting enzyme (ACE) with a more stable bond energy (-6.32 kcal/mol) compared to the standard ACE inhibitor lisinopril (-4.66 kcal/mol). However, further in silico studies are needed to explore the interaction of active compounds in sembung leaves for the development of antihypertensive drugs.

2. Methodology

In silico is a research method that utilizes computing activity and molecular databases. The in silico test is carried out by docking the candidate molecules of the drug compound with the target protein. Molecular Docking is carried out by paying attention to the properties of ligands and target proteins to predict the interaction of two molecules (protein-ligands). The benefits and use of the method, a study was carried out on screening in silico active compounds of splice leaves (Blumea balsamifera DC) as an antihypertensive using two software (Autodock Vina and PyRx). The calculation results from two software to be compared against commercial drugs used as antihypertensives.

The study aims to calculate the interaction of active compound in sembung leaves (Blumea balsamifera DC) to angiotensin-converting enzyme (ACE) related antihypertensive activity in silico with pharmacodynamic and pharmacokinetic parameters.

2.1. Tools and Materials

The tools used in the study are divided into two types: hardware and software. The hardware ASUS laptops with X202E type x64-based, Intel CORE i3 processors, 4 GB of RAM, and the Windows 10 operating system. The software used Visual Molecular Dynamics (VMD) version 1.9.3, Marvin Sketch, Marvin View, AutoDock Tools 1.5.6, Python Molecule Viewer-1.5.6, AutoDock Vina 1.1.2, Chimera 1.13, LigPlot+, Discovery Studio Visualizer, PyRx 8.0 and PyMOL.

2.2. Preparation of Target Protein Structure

The receptor is an ACE enzyme with a code (PDB: 1UZF). The receptor is downloaded from the page (https://www.rcsb.org/search) as a file with the format *.pdb. The structure was selected based on species suitability, the number of mutases[7], and the resolution of ≤ 2Å. Enzyme preparation is carried out using VMD (Visual Molecular Dynamics) software or Discovery Studio Visualizer by removing water molecules and other molecules. The optimization was carried out using AutoDock Tools 1.5.6, increasing the Kollman charge and adding polar hydrogen.

2.3. Preparation of Test And Comparative Ligand Structures (Control)

Test ligands are active compounds of sembung leaves with as many as 18 compounds obtained from the KNApSAcK Core System (http://www.knapsackfamily.com/knapsack_core/top.php) and Ijah Analytics (http://ijah.apps.cs.ipb.ac.id/) sites. Meanwhile, control ligands, namely *captopril*, have been known to inhibit receptors. Control ligands are obtained from the separation of complex 1UZF, while ligan tests can be downloaded on the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) in the form of 3D structures. Some ligands not found in PubChem were drawn using Marvin Sketch software, and all ligands were adjusted at pH 7.4, Furthermore, ligand optimization is carried out using AutoDock Tools 1.5.6 by adding Gasteiger charge and hydrogen and then setting non-polar merge and ligand torque. Ligands that cannot be prepared in AutoDock Tools 1.5.6 are prepared using Chimera 1.13 by minimizing the structure, adding Gasteiger charges, and adding hydrogen.

2.4. Grid Box Validation

Grid box validation is done by attaching natural ligands tethered to proteins (receptors) using Autodock Tools and PyRx software to see the receptor's active site. The grid box in Autodock tools software is set at x= 10, y= 10, z= 12, with the center point coordinates at x= 40.835, y= 34.382, z= 44.607. The grid box in PyRx software is set at x= 10.038, y= 10.006, z=

12.064, with center point coordinates x= 40.745, y= 34.897, and z= 44.154. The validation of the two software uses 1Å spacing with the exhaustiveness value set at 32. Redocking was carried out 20 times until an RMSD (Root Mean Square Deviation) value was obtained < 2Å. The results of redocking were visualized using PyMOL, and the interactions were visualized using LigPlot+ by first combining redocking results and proteins (receptors) using PyMOL.

2.5. Virtual Screening and Molecular Docking

Docking is done with two different software, namely using Autodock and PyRx. Docking with Autodock is done by preparing receptor files and test ligands stored in *.pdbqt form copied and collected in a vina folder (working directory). The name of the receptor, ligand, grid box size, center box, number of modes, and exhaustiveness is written in the Notepad++ application and stored under the name conf.

Analysis of Gibbs-free energy and space interactions

The docking results are side by side, some ligand modes are stored in a file with the format *.pdbqt, and the affinity and RMSD energy information is stored in the log file.txt. Based on these results can be analyzed the values of ΔG, their inhibition costs (Ki), and dissociation constants (K_d) using the formulas $K_i = e^{\left(\frac{\Delta G}{RT}\right)}$ and $K_d = e^{\left(\frac{\Delta G}{-RT}\right)}$

2.6. Lipinski analysis

Lipinski calculation was performed online access on http://www.swissadme.ch/. The Analysis begins by opening the web, then inputting the active compound's smile file (Test ligand). The results of the Lipinski analysis will come out after clicking the run.

2.7. ADME analysis

ADME predictions on compounds are carried out online access on http://biosig.unimelb.edu.au/pkcsm/. ADME analysis using the web is performed by inputting the smile file of the active compound. Then click (ADMET) to obtain the results of the pharmacokinetic analysis.

2.8. Toxicity Analysis of Compounds

Toxicity analysis is carried out using the web (http://biosig.unimelb.edu.au/pkcsm/) by inputting the SMILE file of the active compound on the web server, then clicking (Toxicity) to get the toxicity value of the compounds

3. Result and Discussion

3.1. Preparation of Receptor Structures and Ligands

The complex has several tethered ligands, namely 2 acetylglucosamine chains and 1 captopril chain, and there are three ions, namely 1 zinc ion (Zn^{2+}) and 2 chloride ions (Cl^-) (Fig. 1). However, those that are close to the active site are only captopril and Zn ions (Fig. 1), so at the preparation stage, acetylglucosamine ligands and Cl ions are removed, remaining angiotensin-converting enzyme (ACE), captopril, and Zn ions. The receptor structure used in this study was the result of X-ray crystallography, the structure of ACE in humans with a resolution of 2Å, and the minimum number of mutations (0) listed in the Protein Data Bank (PDB) with the code (PDB: 1UZF).

The protein structure is chosen with a resolution less than or equal to 2Å since high-resolution structures (small resolution values) have fine crystalline details, making it very regular and easy to see each atom at electron density. The preparation stage separates proteins (receptors) that bind Zn ions with their ligands (Fig. 3). This process aims to obtain proteins without their natural ligands and ligands. The receptors are separated using VMD (Visual Molecular Dynamics) and Discovery Studio Visualizer by removing water molecules and other unnecessary molecules, such as ligands and ions bound around the receptor. There is no interference with the analysis and calculation process when conducting Molecular Docking. Adding hydrogen atoms aims to adjust the docking atmosphere to approach the pH atmosphere

in the body. In addition, the addition of hydrogen atoms aims to create the best hydrogen bonds by placing hydrogen atoms, and the functional group does not affect the density of electrons so that interactions are formed.

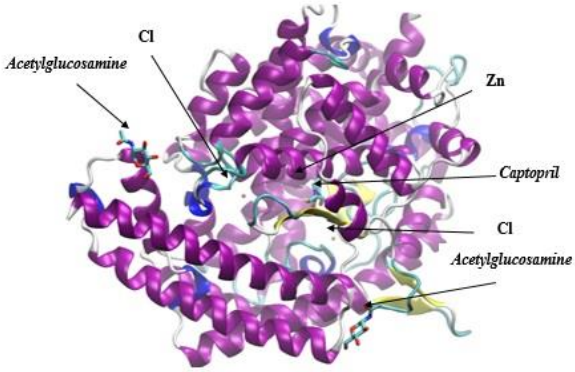

Fig 1. Complex structure (PDB: 1UZF)

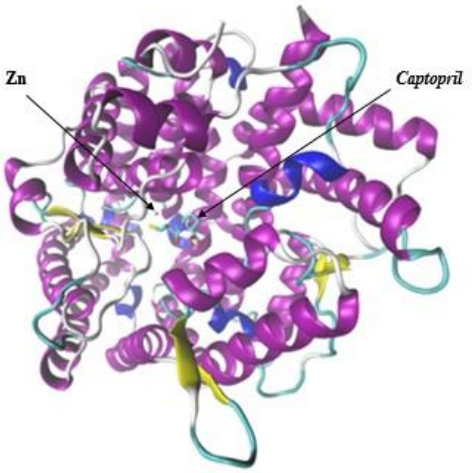

Fig 2. Protein Complex, Ligands, and Zn

The control ligand used in this study was captopril. Captopril is a natural ligand tethered to a complex (PBD:1UZF) (Fig. 4). Test ligands were used for 18 compounds of sembung leaves. The Ligand Control and Ligand tests were prepared using Chimera and Autodock Tools. The interaction between ligands and receptors is enhanced by making ligands flexible to interact with receptors. Additional hydrogen changes in ligands aim to make the docking atmosphere close to the pH atmosphere in the body and have good solubility like its control ligands (captopril).

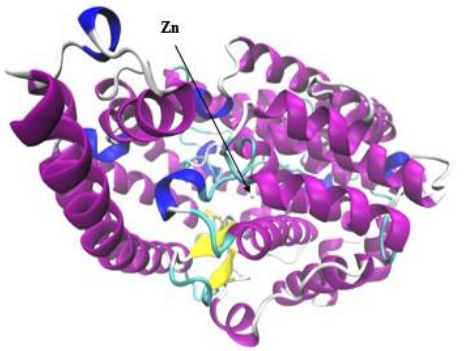

Fig 3. Proteins bind to Zn.

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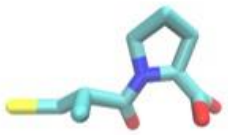

Fig 4. Control Ligand (Captopril)

3.2. Validation Grid Box, Autodock and PyRx

The grid box molecular docking is validated by redocking natural ligands against the target protein using Autodock Tools and PyRx software. The validation carried out is the testing stage of the molecular docking method to ensure that the size and position of the grid box are compatible with the active site enzymes (proteins).

Autodock Vina was validated by redocking natural ligands against receptors 20 times by first setting the grid box in the Autodock Tools software. The grid box size was set at x= 10, y= 10, z= 12 with center point coordinates at x= 40.835, y= 34.382, z= 44.607 using spacing 1Å, and the exhaustiveness value is set at 32. Exhaustiveness is a function that controls accuracy in the minimum global finding. High exhaustiveness value can provide better pose search results. The overlay pose of natural ligand conformation (captopril) and redocking ligands are seen in clusters (Fig. 5) close to the position of natural ligands.

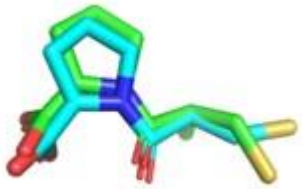

Fig 5. Overlay the position of the natural ligand (blue) and the resulting ligand 2 (green)

The redocking result produced 19 poses. Pose 1 had the most negligible affinity energy value -6.5 kcal/mol. The results showed that captopril binds hydrogen to His-353 (2.95 Å), His-513 (3.10 Å), Tyr-523 (2.83 Å), and Zn (2.82 Å). There is a direct interaction of several amino acids with Zn forming hydrogen bonds, including binding to Glu-411 (2.04 Å), His-383 (2.10 Å), and His-387 (2.11 Å). In addition, six other amino acids were identified as hydrophobic interacting with captopril, namely Gln-281, Glu-384, Phe-457, Phe-527, Thr-282, and Val-380 (Fig. 6). The result is a residue involved in active side binding. According to Natesh *et al.* [8], captopril interacts with hydrogen with Glu-384, His-353, His-513, Lys-511, and Tyr-520, forming hydrogen bonds with Zn, namely with Glu-411, His-383, and His-387.

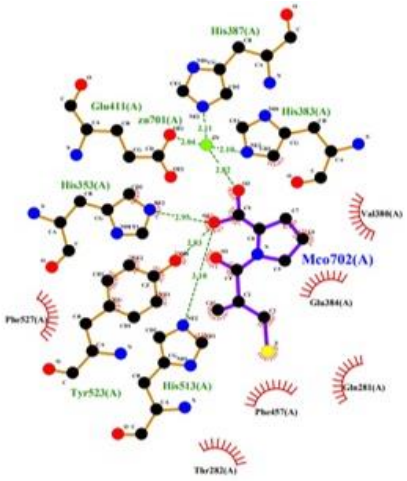

Fig 6. Interaction of *redocking* pose 1 results

PyRx validation is done by first setting the grid box size in the PyRx software. The size of the grid box this set at x= 10.038, y= 10.006, z= 12.064, with the coordinates of the center point x= 40.745, y= 34.897, z= 44.154. Validation uses a spacing of 1Å based on Autodock Vina, with the exhaustiveness value set at 32. The results of the conformation of natural ligands (captopril) and redocking ligands are seen in clusters (Fig. 7) close to the position of natural ligands.

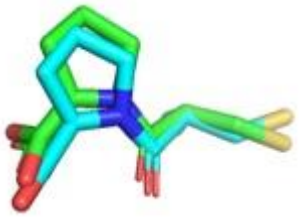

Fig 7. Overlay position of the natural ligand (blue) and ligand from pose 3 (green)

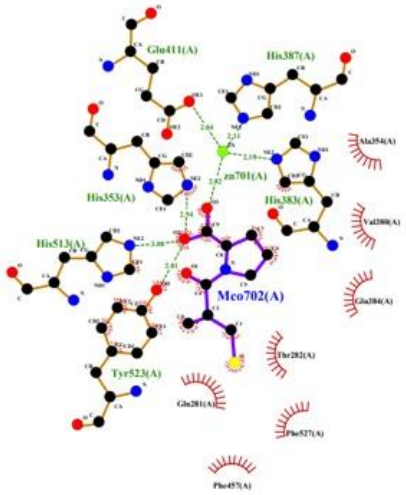

Fig 8. Interaction captopril resulting from redocking pose 1

The redocking result produces 9 poses. Pose 1 has the smallest affinity energy value -6.5 kcal/mol. The results showed that captopril binds hydrogen to Zn (2.82 Å), His-353 (2.94 Å), His- 513 (3.08 Å), and Tyr-523 (2.81 Å). There is a direct interaction of several amino acids with Zn forming hydrogen bonds, including binding to Glu-411 (2.04 Å), His-383 (2.10 Å), and His-387 (2.11 Å). In addition, seven other amino acids were identified as hydrophobic interacting with captopril, namely Ala-354, Gln-281, Glu-384, Phe-457, Phe-527, Thr-282, and Val-380 (Fig. 8). The result has satisfied all the residues involved in the same active site binding as the redocking results using the Autodock. With similarities in the interaction of hydrogen with Zn and the interaction of hydrogen bonds with other residues, and evidenced by the visualization of the results of squeezed redocking, the molecular tethering method can be declared appropriate and used for docking test ligands.

3.3. Molecular Docking

Molecular docking in this study was carried out to find the potential of active sembung leaves as ACE inhibitors by comparing the results with control compounds (captopril) which are commercial ACE inhibitors using AutoDock Vina and PyRx software. Trott and Olson [9] stated that the vina autodock has a high speed and accuracy, so it is very suitable for molecular docking. PyRx is a virtual screening software to discover drug candidates from potential compounds (ligands). The selection of PyRx software is based on the fact that PyRx has several methods based on one software, including the Vina-based PyRx method. According to Saputri et al. [10], PyRx-Vina has the advantage of a low error rate, accuracy, and ease of operation.

Docking was performed on all sembung active leaf compound test ligands to identify and obtain the best ligands based on the ΔG between the ligands and receptors. The two docking software are used to determine the accuracy of the docking that has been done by comparing the results. The bond-free energy (ΔG) redocking control ligands (captopril) obtained from the validation results was -6.5 kcal/mol. Based on this, the test ligands that passed as the best test ligands from the docking results using Autodock Vina and PyRx, namely those whose results both in the results of Autodock Vina or PyRx had a value of ΔG ≤-6.5 kcal/mol.

The docking results of two software identified 10 ligan that passed had a low ΔG value than the control ligand, sorted from the lowest, namely (-)-Blumealactone C, 3,5,7,2-Tetrahydroxy-5-methoxy flavanone, Luteolin, (-)-Blumealactone A, (-)-Blumealactone B, Blumeatin, Taxifolin 4- methyl ether, 3,5,2-Trihydroxy-7,5-dimethoxy flavanone, Quercetin, and Tamarixetin (Table 1). The best ligands have the lowest binding free energy.

3.4. Pharmacodynamic Analysis

Pharmacodynamics includes analyzing the results of tethering compound molecules with RMSD values ≤ 2 Å, Analysis of free energy values, inhibition constants, dissociation constants, and their interactions. Knowledge of structural data and protein-ligand bond interactions identified to optimize the process of discovering new drugs and a deep understanding of the properties of molecular interactions is also of great importance in facilitating the discovery and development of drugs.

Table 1. Molecular docking result (Autodock Vina and PyRx)

No	Name of Compound	Affinity energy (kcal/mol)	Affinity energy (kcal/mol)
		Autodock Vina	***PyRx***
1	(-)-Blumealactone A	-7.4	-7.0
2	(-)-Blumealactone B	-7.4	-6.5
3	(-)-Blumealactone C	-8.3	-7.1
4	(-)-Borneol	-5.9	-5.8
5	3,4-Dihydroxybenzoic acid	-5.7	-6.0
6	3,5,2-Trihydroxy-7,5-dimethoxyflavone	-6.6	-6.3
7	3,5,2-Trihydroxy-7,5-dimethoxyflavanone	-6.5	-6.5
8	3,5,7,2-Tetrahydroxy-5-methoxyflavanone	-7.8	-8.0
9	Blumeatin	-7.0	-7.1
10	Dihydrotamarixetin	-6.4	-6.5
11	Luteolin	-7.6	-7.5
12	Quercetin	-6.5	-6.5
13	Rhamnetin	-6.2	-6.2
14	Tamarixetin	-6.5	-6.5
15	Taxifolin 4-methyl ether	-6.7	-6.8
16	Taxifolin 7,3-dimethyl ether	-6.2	-6.1
17	Taxifolin 7,4-dimethyl ether	-6.3	-6.2
18	Xanthoxylin	-6.1	-6.1

[a] Description: = ligands that have the potential to be antihypertensive

3.5. Analysis of bond-free energy and interaction of ligan to receptors

The Vina and PyRx autodocks produce output from affinity energy (kcal/mol) and RMSD. Both software does not provide the value of the inhibition constant and the dissociation constant, so calculating the value of Ki requires the formula: Ki = $e^{(\frac{\Delta G}{RT})}$ [11] and to calculate the value of the dissociation constant, it is necessary to use the equations: ΔG = −RT In Kd (Moreno-Incandescent 2011), with the value of ΔG in units (cal/mol), gas constants (R= 1.986 cal/mol) and temperature (T= 25°C = 298 K).

The docking results in these two software analyzed the value of bond-free energy (ΔG), inhibition constant (Ki), dissociation constant (Kd), and its association interaction. Binding affinity is the strength of the crucial interaction between a protein and its ligand or binding pair. The parameters of conformational stability between ligands and receptors can be seen from the value of ΔG. Ligan-receptors that interact with each other will tend to be at the lowest energy state; this condition causes the molecule to be stable. Based on the study's results, 10 test ligands had a lower ΔG close to the control ligand ΔG, which was -6.5 kcal/mol. The 10 test ligands potentially suggest antihypertensives candidates (Tables 2 and Table 3).

The inhibition constant (Ki) is a value that indicates the magnitude of the resistance between the ligand and the receptor. The value is directly proportional to Gibbs's free energy (ΔG). According to Tambunan and Alamudi [12], the inhibition constants are getting smaller, indicating that the complexes formed between ligands and standards are pretty strong, caused by the increased torsional energy of the complex, thereby making the receptors and ligands stable. The simulation calculations on the two software showed that the ten ligands had inhibition activity. Based on the results of the two software, both of which have a Ki value smaller than the control ligand value (Captopril) of 16.99 μM, there are 6 test ligands, namely (-)-Blumealactone A, (-)-Blumealactone C, 3,5,7,2-Tetrahydroxy-5- methoxy flavanone, Blumeatin, Luteolin, and Taxifolin 4-methyl ether. The compound is predicted to have a better inhibitory potential than Captopril (Table 2 and Table 3).

The dissociation constant (Kd) is inversely proportional to the receptor's affinity to the ligand, so the higher s Kd, the lower the ligand's affinity to the receptor. The simulation calculations on the two software showed that the 10 ligands strongly bonded with the receptor. The results of both software have a Kd value more significant than the control ligand (Captopril) of 16.99 μM. There are 6 test ligands, namely (-)-Blumealactone A, (-)-Blumealactone C, 3,5,7,2-Tetrahydroxy-5-methoxyflavanone, Blumeatin, Luteolin, and Taxifolin 4-methyl ether. The compound is predicted to have stronger bonds/interactions than Captopril (Table 2 and Table 3). The simulation's minor errors were proven by analyzing the interactions formed on ligands-receptors.

Analysis of the interaction of amino acid residues aims to identify the interactions between ligands and receptors. Hydrogen bonds are formed by a hydroxyl group, an amide group (peptide bond), or any other group that can be a donor or acceptor of hydrogen bonds. According to Nursamsiar et al. [13], a hydrogen bond is an interaction that can stabilize ligand bonds with receptors. Meanwhile, hydrophobic interaction is formed between molecules that have non-polar properties. According to Arwansyah et al. [14], hydrophobic interaction also plays a role in determining the stability of ligands against receptors.

Angiotensin-converting enzyme (ACE) is predicted to have three active sites, namely S1, S2, and S1′ (Fig. 9). The amino acid residues that include S1 are Ala-354, Glu-384, and Tyr-523, the amino acid residues that form S2 are Gln-281, His-353, His-513, Lys-511, and Tyr-520, while the amino acid residues that include S1′ are Glu-162. ACE, an enzyme of the zinc-metalo proteinase group, has zinc ions (Zn2 +) where the active side binds to Glu-411, His-383, and His-387. Zinc is an essential component of ace catalytic binding sites. Control ligands (Captopril) are competitive inhibitors that can make direct interactions with catalytic Zn2+ ions. Four amino acid residues are located in the catalytic side area, namely Glu-384, Glu-411, His-383, and His-387.

Amino acid residues interacting with Zn are essential in stabilizing conformations and catalytic interactions of enzymes to the substrate. Captopril is bound to the central carbonyl group and the proline carboxylate group. The leading carbonyl group between the sulfhydryl group and the proline terminal is positioned by two strong hydrogen bonds of two histidines (His-513 and His-353). Similarly, one oxygen from the carboxylate group of the proline part interacts with Tyr-520, Gln-281, and Lys-511. This interaction acts as a backstop, positioning the substrate molecules so that the carbon from the scissile bond can undergo nucleophilic attacks. A scissile bond is a covalent chemical bond that enzymes can break. According to Wang et al. [15], the nucleophilic attack was accompanied by the displacement of protons to Glu-384. Such interactions allow the formation of tetrahedral intermediates. The resulting tetrahedral

intermediate is stabilized by oxyanion holes provided by zinc ions, Tyr-523 and His-513. The oxyanion hole is an enzyme-active sited bag that stabilizes the negative charge of the transition state on deprotonated oxygen or alkoxides.

Analysis based on the ACE inhibition mechanism was carried out by identifying ligands that had interactions with metal ions (Zn2+) and hydrogen interactions on the active side. The results showed that ligands interacting with Zn have an ideal binding potential because they meet the entire catalytic side. The interaction of docking results using Autodock vina identified as having interactions with Zn was met by Captopril (control ligand) and two test ligands 3,5,7,2-Tetrahydroxy-5-methoxyflavanone and Luteolin (Table 2). Meanwhile, ligands that do not bind to Zn but have a hydrogen bond on the active side are identified to have ACE inhibition activity which is not much different from ligands

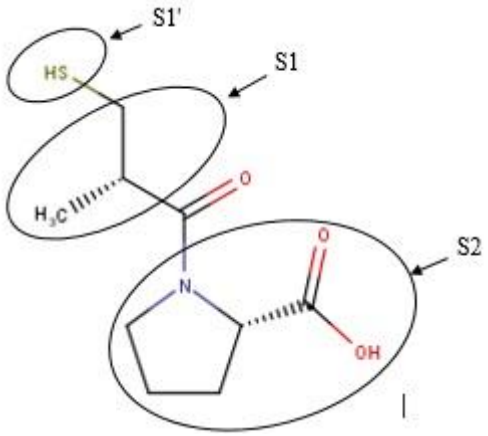

Fig 9. 2D structure *of captopril* (control ligand)

that have interactions with Zn because the interaction of hydrogen on the active side also affects the interaction of ligands to enzymes (ACE). The results showed that seven ligands had hydrogen interactions but had no interactions with Zn, namely (-)-Blumealactone A, (-)-Blumealactone B, 3,5,2-Trihydroxy-7,5-dimethoxyflavanone, Blumeatin, Quercetin, Tamarixetin, and Taxifolin 4- methyl ether (Table 2). The identified ligan test has no interaction with Zn and no hydrogen bond on the active side. It is unclassified as an ACE inhibitor because it has no potential to inhibit ACE. One ligand has no hydrogen bond, namely (-)-Blumealactone C (Table 2).

The *docking* results using PyRx identified as having interactions with Zn were met by Captopril (control ligands) and three test ligands, namely 3,5,7,2-Tetrahydroxy-5-methoxyflavanone, Blumeatin, and *Luteolin* (Table 3). Meanwhile, ligands that do not bind to Zn but have hydrogen bonds on the active side are identified as having ACE inhibition activity. There are six ligands, namely (-)-Blumealactone A, (-)-Blumealactone B, 3,5,2-Trihydroxy-7,5-dimethoxyflavanone.

Table 2. Bond-free energy and ligand interaction of Vina Autodock docking results

Compound Name	ΔG (kcal/ mol)	Ki (μM)	Kd (μM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
Captopril (Control ligand)	-6.5	16.99	5.89×10^{10}	Glu-411 His-383 His-387	His-353 His-513 Tyr-523 Zn-701	Glu-281 Glu-384 Phe-457 Phe-527 Thr-282 Val-380
(-)-Blumealactone A	-7.4	3.71	2.69×10^{11}	-	Gln-281 Lys-511 Thr-282	Ala-354 Asn-277 Glu-376

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Compound Name	ΔG (kcal/ mol)	Ki (μM)	Kd (μM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
						His-353 His-383 His-513 Tyr-520 Tyr-523 Val-380
(-)-Blumealactone B	-7.4	3.71	2.69×10^{11}	-	Gln-281 Lys-511	Ala-354 Asp-415 Asp-453 Glu-376 His-353 His-383 His-513 Phe-457 Phe-527 Thr-282 Tyr-520 Tyr-523 Val-380
(-)-Blumealactone C	-8.3	0.81	1.23×10^{12}	-	-	Asp-415 Asp-453 Gln-281 His-383 His-513 His-523 Phe-257 Phe-527 Trp-279 Tyr-520 Tyr-523 Val-380
3,5,2-Trihydroxy-7,5-dimethoxyflavanone	-6.5	16.99	5.89×10^{10}	-	Ala-354 Ala-356 His-353 His-513	Asp-415 Glu-384 Glu-411 His-383 His-387 Phe-527 Ser-355 Ser-526 Tyr-523 Val-380
3,5,7,2-Tetrahydroxy-5-methoxyflavanone	-7.8	1.89	5.29×10^{11}	Glu-411 His-383 His-387	Gln-281 Glu-384 His-383 His-513 Zn701	Glu-376 His-353 Phe-527 Tyr-523 Val-380
Blumeatin	-7.0	7.30	1.37×10^{11}	-	His-353	Ala-354 Glu-384 Glu-411 His-383 His-387 Phe-512 Ser-355 Tyr-523 Val-380 Val-518
Luteolin	-7.6	2.65	3.78×10^{11}	Glu-411 His-383 His-387	Gln-281 His-353 His-387 His-513 Lys-511 Tyr-520	Ala-354 Glu-384 Phe-457 Phe-512 Ser-355 Tyr-523

Compound Name	ΔG (kcal/ mol)	Ki (µM)	Kd (µM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
Quercetin	-6.5	16.99	5.89×10^{10}	-	Zn-701 Ala-354 Glu-384	Val-518 His-353 His-387 His-513 Phe-457 Phe-527 Ser-355 Tyr-523
Tamarixetin	-6.5	16.99	5.89×10^{10}	-	Ala-354 Ala-356	Glu-384 His-353 His-387 His-513 Phe-457 Phe-527 Ser-355 Tyr-523
Taxifolin 4-methyl ether	-6.7	12.12	8.25×10^{10}	-	Ala-354	Glu-384 His-353 His-387 His-513 Phe-457 Phe-527 Ser-355 Tyr-523

[b.] Description = Catalytic residue

Quercetin, Tamarixetin, and Taxifolin 4-methyl ether (Table 3). The identified test ligands have no interaction with Zn and no hydrogen bond on the active side, not including ace inhibitors and one ligand, namely (-)-Blumealactone C (Table 3). Analysis of the interaction of ligands from Docking using Autodock vina and PyRx identified nine ligands that could potentially inhibit ACE with a value of ΔG and Ki, which was lower or equal to the control ligand (captopril) and a Kd value greater than or equal to the control ligand. The nine test ligands carried out a pharmacokinetic analysis of an oral drug prerequisite when administrated until it came out through the human body's excretory organs.

Table 3. Bond-free energy and ligand interactions resulting from PyRx docking

Compound Name	ΔG (kcal/ mol)	Ki (µM)	Kd (µM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
Captopril	-6.5	16.99	5.89×10^{10}	His-383 His-387 Glu-411	His-353 His-513 Tyr-523 Zn-701	Ala-354 Glu-281 Glu-384 Phe-457 Phe-527 Thr-282 Val-380
(-)-Blumealactone A	-7.0	7.30	1.37×10^{11}	-	Gln-281 Lys-511	Ala-354 Asp-415 Asp-453 His-353 His-383 His-513 Phe-457 Phe-527 Thr-282 Tyr-520 Tyr-523 Val-380
(-)-Blumealactone B	-6.5	16.99	5.89×10^{10}	-	Gln-281 Lys-511	Ala-354 Asp-415 Asp-453

Compound Name	ΔG (kcal/ mol)	Ki (μM)	Kd (μM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
(-)-Blumealactone C	-7.1	6.16	1.62×10^{11}	-	-	Glu-384 His-353 His-383 His-513 Phe-457 Phe-527 Thr-282 Tyr-520 Tyr-523 Val-380 Asp-415 Asp-453 Gln-281 His-353 His-383 His-513 Lys-511 Phe-457 Phe-527 Tyr-520 Tyr-523 Val-380
3,5,2-Trihydroxy-7,5-dimethoxyflavanone	-6.5	16.99	5.89×10^{10}	-	Ala-354 Tyr-523	Asp-453 Glu-376 Glu-384 Glu-411 His-353 His-383 Phe-457 Phe-527 Thr-282 Val-380
3,5,7,2-Tetrahydroxy-5-methoxyflavanone	-8.0	1.35	7.42×10^{11}	Glu-411 His-383 His-387	Gln-281 Glu-384 His-383 His-513 Zn-701	Asp-453 Glu-376 His-353 Phe-527 Tyr-523 Val-380
Blumeatin	-7.1	6.16	1.62×10^{11}	Glu-411 His-383 His-387	His-387 Zn-701	Ala-354 Asp-415 Glu-384 His-353 His-513 Phe-512 Phe-527 Tyr-523 Val-380 Val-518
Luteolin	-7.5	3.14	3.19×10^{11}	Glu-411 His-383 His-387	Gln-281 Glu-384 His-387 Tyr-520 Zn-701	Ala-354 Asp-415 His-353 His-513 Phe-457 Phe-527 Ser-355 Tyr-523
Quercetin	-6.5	16.99	5.89×10^{10}	-	Ala-354 His-387	Glu-384 Glu-411 His-353 His-383 His-513 Phe-457

Compound Name	ΔG (kcal/ mol)	Ki (μM)	Kd (μM)	Interaction with Zn	Hydrogen Bonds	Hydrophobic Bonds
Tamarixetin	-6.5	16.99	5.89×10^{10}	-	Ala-354	Phe-527 Tyr-523 Val-380 Glu-384 His-353 His-383 His-387 His-513 Phe-457 Phe-527 Ser-355 Tyr-523
Taxifolin 4-methyl ether	-6.8	10.23	9.77×10^{10}	-	Ala-354 Glu-384	His-353 His-383 His-387 His-513 Phe-457 Phe-527 Tyr-523 Ser-355

[c.] Description: = Catalytic residue

3.6. Pharmacokinetics Analysis

The pharmacokinetic Analysis includes physicochemical properties assay with Lipinski's Rule of Five, ADME assay with Human Intestinal absorption (HIA) value parameters, steady-state volume of distribution (Vdss), CYP2D6 Inhibitor, CYP2D6 Substrate, Total clearance, Renal OTC2 and toxicity test with mutagenic, hepatotoxic, skin sensitization as well as acute LD_{50} (Lethal Dose) parameters. Generally, a promising drug candidate must have a specific protein already absorbed, distributed, metabolized, and excreted (absorption, distribution, metabolism, and excretion) and have a low level of toxicity (LD_{50}).

Analysis of the nature of ligands based on the Lipinski rule

The Lipinski rule is used to determine a ligand's physicochemical properties in determining a compound's hydrophobic/hydrophilic character when it crosses the cell membrane by passive diffusion. Prediction Lipinski is essential as one of the parameters for identifying a drug candidate intended for oral use. It is common for a drug to be administered orally if it does not violate more than one Lipinski rule. The rules include (1) the number of hydrogen bond donors not more than 5 (O-H and N-H groups), (2) the number of hydrogen bond acceptors not more than 10 (nitrogen and oxygen atoms), (3) molecular weight not greater than 500 daltons, (4) lipophilicity (log P) not greater than 5 [16] and (5) molar refractivity (RM) should range from 40-130.

Lipophilicity is expressed as the ratio of octane solubility to water solubility as log P indicates the solubility coefficient in fat or water with a range of -0.4 to 5. The value of log P is more negative and not good because the molecule cannot pass through the lipid membrane of the bilayer [17]. While log values P > 5 have a significant permeation potential to cross the membrane and have a high level of toxicity as similarity on the molecule hydrophobic properties causing selectivity of ligand bonds to the target molecule to be reduced.

The number of hydrogen bond donors and acceptors describes the higher the hydrogen bond capacity, the higher the energy required for absorption. Hydrogen bonds can establish essential interactions with the target molecule. Molar refraction is a steric parameter influenced by the spatial arrangement of the aromatic ring of the compound. The molar refraction value is the total value of the polarisability of drug molecules highly dependent on temperature, refractive index, and pressure [18].

Based on the docking results, all active compounds of sembung leaves escaped and were predicted to have a good potential of nine compounds. Ligan tests that passed the Analysis showed no deviations in the Lipinski rule (Table 4), so it can be proven that the test ligands have good bioavailability and can be used in subsequent analyses.

Table 4. Results of the Analysis of ligand properties

Compound Name	Log P	Molecular Weight (g/mol)	H Bond Donor	H Bond Akseptor	Molar Refractivity
(-)-Blumealactone A	2.44	364.43	1	6	95.82
(-)-Blumealactone B	2.58	364.43	1	6	95.82
3,5,2-Trihydroxy-7,5-dimethoxyflavanone	1.37	332.30	3	7	83.69
3,5,7,2-Tetrahydroxy-5-methoxyflavanone	1.04	318.28	4	7	79.22
Blumeatin	1.88	302.28	3	6	78.06
Luteolin	1.73	286.24	4	6	76.01
Quercetin	1.23	302.24	5	7	78.03
Tamarixetin	1.85	316.26	4	7	82.50
Taxifolin 4-methyl ether	1.07	318.28	4	7	79.22

ADME analysis

ADME analysis (absorption, distribution, metabolism, and excretion) is crucial in designing an oral drug. ADME analysis is carried out to determine whether the compounds used as oral drugs can be appropriately distributed to the target tissue and are metabolized and excreted correctly in the body. Analysis of ADME (absorption, distribution, metabolism, and excretion) in the study was carried out with parameter HIA (Human Intestinal Absorption), VDss (Steady State of Volume Distribution), CYP2D6 Inhibitor (Cytochrome P2D6 inhibitor), CYP2D6 substrate, Total clearance (CLTOT) and renal organic cation transporter 2 (OCT2).

Table 5. Results of prediction of pharmacokinetic properties (ADME)

Compound Name	HIA (%)	VDss (log L/ kg)	CYP2D6 substrate	CYP2D6 Inhibitor	Renal OTC2	CLTOT (log ml /min/kg)
(-)-Blumealactone A	97.336	-0.003	No	No	No	1.271
(-)-Blumealactone B	97.33	0.011	No	No	No	1.183
3,5,2-Trihydroxy-7,5-dimethoxyflavanone	94.892	0.084	No	No	No	0.417
3,5,7,2-Tetrahydroxy-5-methoxyflavanone	93.225	0.514	No	No	No	0.364
Blumeatin	74.489	-0.008	No	No	No	0.51
Luteolin	75.17	0.739	No	No	No	0.452
Quercetin	68.36	0.842	No	No	No	0.382
Tamarixetin	72.625	0.509	No	No	No	0.447
Taxifolin 4-methyl ether	79.978	0.556	No	No	No	-0.041

The analysis results (Tabel 5) showed that HIA (Human Intestinal Absorption) of all compounds contained in the splice leaves ranged from 68.36% to 97.336%, meaning that there was good absorption in the human intestine, according to Hou *et al.* [19] showed that the compound has a good absorption value if it reaches more than 30%. The higher the value of

VDss, the more drugs are distributed in the tissues than in plasma. VDss is considered low if below 0.71 L/kg (VDss logs < -0.15) and high if above 2.81 L/kg (VDss logs > 0.45). The results of the VDss values of all sembung leaf compounds range from -0.008 to 0.842 Log L/kg (Table 5) and can be well distributed in the blood plasma.

Predicting metabolic processes is how drugs will be changed in the body to form metabolites. The enzyme cytochrome P450 (CYP) carries most small molecule drug metabolism in the liver. Cytochrome P450 catalyzes various organic chemical reactions in the metabolism of drugs. The results of the predictions showed that the compound did not affect or inhibit the enzyme CYP2D6 (cytochrome P450 isoenzyme) (Table 5). Drugs that inhibit the CYP enzyme pathway can cause an increase in the concentration of other drugs metabolized by the same pathway, which can result in drug toxicity.

The compound excretion process is predicted by measuring CLTOT and OCT2 settings. The results of the prediction of OCT2 (Table 5) show that the test compound is not an OCT2 renal substrate, so if the compound is made, oral drugs consumed together with renal OCT inhibitors do not cause a toxic effect. The predicted results of the total clearance value (Total 5) of the spongy leaf compound range from -0.041 to 1.271 ml/min/kg. This value shows the excretion rate of the spongy leaf compound from low to large.

Analysis of Ligand Toxicity

Toxicity prediction is a crucial thing that must be done in a drug design. Toxicity is a measure that describes the ability of a compound to cause cell or organ damage to an organism. Toxicity prediction in this study was carried out using four parameters: Ames Ames toxicity, Hepatotoxicity, Skin Sensitisation, dan Oral Rat Acute Toxicity (LD_{50}).

Ames toxicity is a method used to assess the potential of mutagenic compounds using bacteria. The prediction results (Table 6) show seven compounds of sembung leaves are not mutagenic, and one compound, namely (-)-blumeatin, is identified as a mutagenic Hepatotoxicity. The hepatotoxicity test is used to determine the ability of compounds to cause damage to liver organs. The results of the predictions (Table 6) identified that all ligands did not have the potential to trigger liver damage.

Table 6. Results of prediction of pharmacokinetic properties (Toxicity)

Compound Name	Ames toxicity	Hepatotoxicity	Skin Sensitisation	Oral Rat Acute Toxicity (LD50) (mol/kg)
(-)-Blumealactone A	No	No	No	3.113
(-)-Blumealactone B	No	No	No	3.113
3,5,2-Trihydroxy-7,5-dimethoxyflavanone	No	No	No	1.673
3,5,7,2-Tetrahydroxy-5-methoxyflavanone	No	No	No	1.815
Blumeatin	Yes	No	No	2.169
Luteolin	No	No	No	2.496
Quercetin	No	No	No	2.535
Tamarixetin	No	No	No	2.432
Taxifolin 4-methyl ether	No	No	No	1.84

[d.] Description: = Not a good criterion for oral drugs

Skin Sensitisation assays predict whether certain compounds can cause allergic contact dermatitis. The results of the predictions (Table 6) identify that all ligands do not cause sensitization in the skin. Lethal Dose (LD_{50}) is a dose that can cause death to 50% of the test animal group. According to research (Ferrari and Mario 2022), LD_{50} results of less than 2 mol/kg are highly toxic. The prediction results (Tabel 6) showed that three compounds had high toxicity, namely 3,5,2-Trihydroxy-7,5-dimethoxyflavanone (1.673 mol/kg), 3,5,7,2-Tetrahydroxy-5-methoxyflavanone (1.815 mol/kg), and Taxifolin 4-methyl ether (1.84 mol/kg).

Based on pharmacodynamic and pharmacokinetic Analysis, five active compounds of sembung leaves have promising candidates as hypertension drugs. The series of decreasing potential are Luteolin, (-)-Blumealactone A, (-)-Blumealactone B, Quercetin, and Tamarixetin. Luteolin is the best test ligand with ACE inhibition activity with strong bond interaction because it has a smaller ΔG and Ki than the control ligand (Captopril) and a higher Kd value than the control ligand (Captopril). Furthermore, it demonstrates interaction with Zn, hydrogen bonding on the active site, and meets the criteria in the Analysis of Lipinski, ADME, and toxicity, indicating its safety for use as an oral drug, surpassing the effectiveness of the control ligand (Captopril).

4. Conclusion

Screening *in silico* 18 ligands test of active compounds of sembung leaves carried out using two docking software (autodock vina and PyRx) with pharmacodynamic and pharmacokinetic parameters, it can be identified that there are five active compounds of sembung leaves that have the potential as hypertension drug candidates, namely Luteolin, (-)-Blumealactone A, (-)-Blumealactone B, Quercetin, and Tamarixetin. Luteolin is predicted to be the highest potential ligand as an ACE enzyme inhibitor, with ΔG and Ki values of both software lower than the values of ΔG and Ki of control ligands. Both software's Kd values are higher than the control ligands' Kd values. The ΔG, Ki, and Kd values of Autodock Vina results were 7.6 Kcal/mol, 2.65 μM, and 3.78×10^{11} μM, respectively. The ΔG, Ki, and Kd values of PyRx results were 7.5 Kcal/mol, 3.14 μM, and 3.19×10^{11} μM, respectively. In addition, Luteolin was identified as having an interaction with Zn and having hydrogen bond interactions on the active side and meeting the criteria in the Analysis of Lipinski, ADME, and toxicity. Hence, this compound is relatively safe to use as a drug candidate for treating hypertensive diseases

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