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Research Article

Antihypertension Activity Test of Red Ginger (*Zingiber Officinale* Var. *Rubrum* Roscoe) Ethanol Extract by In Silico Method

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Abstract: Hypertension or high blood pressure is a condition when there is an increase in blood pressure above the normal threshold (> 140/90 mmHg). The bioactive compounds in red ginger are dominated by the terpene group which could inhibit the action of Angiotensin Converting Enzyme (*ACE*-inhibitor). The interaction between *ACE*-inhibitory peptides can be predicted by the in-silico method. The in-silico method was used to predict the interaction and binding energy between bioactive compounds in red ginger ethanol extract (ligand) and *ACE* protein (receptor) which acts as an antihypertensive. The results of GC-MS obtained as many as 5 compounds namely zingiberene, farnesene, ß -sesquiphellandrene, alpha-curcumene and trans-beta-farnesene. The docking results showed the lowest binding energy values for each compound sequentially for *ACE*-trans beta farnesene, *ACE*-alpha curcumene, *ACE*-zingiberene, *ACE*-farnesene, *ACE*-beta sesquiphellandrene are -5.14 kcal/mol, -5.61 kcal/mol, -6.20 kcal/mol, -5.66 kcal/mol, and -6.55 kcal/mol respectively. Based on these results, the lowest bond energy among the 5 compounds was -6.55 kcal/mol at *ACE*-beta sesquiphellandrene docking, so the red ginger ethanol extract can be proposed and tested further as a clinical candidate for antihypertensive drugs.

Keywords: Antihypertensive, red ginger, in silico method

1. INTRODUCTION

Hypertension or known as high blood pressure is a condition where there is an increase in blood pressure above the normal threshold (>140/90 mmHg) [1]. According to the Ministry of Health (2013) hypertension is the third cause of death after stroke and tuberculosis, where the proportion of this death reached 6.7% of the population mortality at all ages in Indonesia [2]. The result of Basic Health Research (Riskesdas) Balitbangkes in 2018 showed the prevalence of hypertension nationally reached 25.8% in 2013 and then rose to 34.11% in 2018 [3].

The proportion of medication-taking recommended by doctors (synthetic drugs) in patients with hypertension in Indonesia was 54.4%, while 45.6% are not routine and they don't take medication recommended by doctors (synthetic drugs). The reason for patients not routinely and not taking medicine recommended by doctors is that 14.5% of patients choose to take traditional medicine and 4.5% cannot stand with side effects of drugs recommended by doctors [3]. Treatment of hypertension using these drugs costs a lot of money because they are used for long-term therapy, even for life, so that it has the potential to cause side effects.

The various side effects due to the use of drugs and the duration of treatment have caused patients to consume medicines derived from herbs or nature, because it is considered that medicines from natural ingredients are safer for the long term than synthetic drugs even though these drugs have not been proven scientifically. Clinically, this belief is only based on the empirical experience of as well as an inheritance from ancestors. In accordance with the developments and demands of the

times, traditional medicines are expected to be able to develop into a class of phytopharmaca drugs that have absolute requirements in terms of quality assurance, efficacy, and safety [4].

Red ginger plants are also widely believed and used by the community dicine. It contains secondary metabolites such as flavonoids, alkaloids, terpenoids, phenols and essential oils. Many studies prove that it has pharmacological activities such as antimicrobial, antioxidant, therapeutic agent, antimutagenic and anti-cancer, antihyperlipidemic, and antihypertensive [5].

The bioactive compounds in red ginger are dominated by the terpene group which can inhibit the work of Angiotensin Converting Enzyme (ACE-inhibitor). Interactions between peptides that are ACE-inhibitors can be predicted by in silico method. In silico is currently well developed in the process of drug discovery from plants to become phytopharmaca drugs [6]. The main aim of this work is to predict the interaction and binding affinity of bioactive compounds in red ginger ethanol extract (ligand) with protein ACE (receptor) which acts as antihypertensive.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The main ingredients used in this study, namely 5 kg red ginger rhizome. The material for extraction is 96% ethanol. The tools used include oven, blender, Whitman filter paper, beaker glass, erlenmeyer, funnel, spatula, rotary evaporator, bread scale, analytical scale, aluminum foil, GC-MS. The device used is a laptop with specifications ® Core ™ i5-7200U CPU @ 2.5Ghz, 8GB RAM, 64-bit Intel operating system, x64-based processor, and software namely Windows 10 Pro, Way2drug, SwissADME, Pymol, AutoDockTools, Biovia Discovery Studio Visualizer.

2.2. Red Ginger Extraction and Phytochemical Testing

The manufacture of ginger powder (simplicia ginger) is made from fresh ginger which is weighed as much as 5 kg then were cut and dried for 4 days at 55-60°C and mashed with a blender. The result obtained is ginger powder. The extraction method used in this research is maceration. 96% ethanol is added with a ratio of ginger powder: ethanol of 1: 4. Then it is tightly closed and left for 24 hours while stirring occasionally. The ethanol was evaporated using a rotary evaporator at a speed of 70 rpm and a temperature of 55°C for 2 hours. Phytochemical tests were carried out by the method GC-MS injector (ShimadzuQP-5050A) using a DB-5 MS column (dimensions 0.25 M × 30 m) and Helium carrier gas at a flow rate of 42 mL/min. The injector temperature is 80°C and the detector temperature is 250°C, while the column temperature used is the programmed temperature, starting with 80°C for 5 minutes and then slowly changing it with a temperature increase of 5°C/min until the temperature reaches 250°C (constant) within 45 minutes. The mass spectrometer condition is an ionization energy of 70 eV, the ionization mode is EI, a split ratio: 24.9, and the detection area is 40-500 m/z. Each peak that appears in the total ion chromatogram is identified by analyzing and comparing the results of the spectrum mass obtained with the mass spectrum in the MS index library.

2.3. Compound Screening

Screening of active compounds produced by GC-MS by entering the name canonical SMILES via PubChem (https://pubchem.ncbi.nlm.nih.gov) on Way2Drug then potentially antihypertensive compounds with Pa> 0.3 were selected (http://www.pharmaexpert.ru/pass*online*).

2.4. Docking Experiments

Docking experiments were done using Autodock Tools 4.2. ACE crystallography structure was obtained from Protein Data Bank (PDB ID: 1O8A). Each ligand's structure were downloaded by PubChem in 3D SDF. Preparation of receptor and ligand use Pymol software to remove waters, native ligand sequences and then export file to PDB format. ACE was prepared by the addition of hydrogen atoms and kollman charges using Autodock Tools. The five ligands were prepared by addition of computing gasteiger, the addition of hydrogen in the non-polar side, and to take into account the

rotatable bonds as well as the protein using Autodock Tools either. Receptor and ligands saved as PDBQT format. The size of the grid box depends on the center of the ligand. After that for search parameter in docking tab, select genetic algorithm, for column number of GA runs write 100, population size writes 150, maximum number of evals write 2500000 and for output is Lamarckian GA(4.2).

3. RESULTS AND DISCUSSION

3.1. Red Ginger Extraction and Phytochemical Testing

3.1.1. Red Ginger Extraction

The macerate of ginger powder (simplicia ginger) was then evaporated using a rotary evaporator at a temperature of 55°C, then the sample was tested with GC-MS.

3.1.2. Phytochemical Testing

Gas Chromatography-Mass Spectrometry (GC-MS) is a combination of gas chromatography and mass spectrometry. GC can be used to separate volatile compounds and for semi-volatile compounds with good resolution and MS can properly identify these compounds along with the most information contained in a compound. One type of compound that is good to be identified using GC-MS is essential oil. Essential oils are volatile compounds [7].

Table 1. Results of GC-MS Ethanol Extract of Red Ginger

No	Time Retention (min)	Formula Compound	Molecular Weight (g/mol)	Name Compound
1	15,842	C15H24	204	Trans-beta-farnesene
2	16 642	C15H22	202	Alpha-Curcumene
3	16 913	C15H24	204	Zingiberene
4	17 243	C15H24	204	Farnesene
5	17 660	C15H24	204	Beta-Sesquiphellandrene

Based on the GC-MS analysis (data not shown), the ethanolic extract of red ginger contains the highest chemical compounds in the third peak, namely zingiberene (45.99%), followed by farnesene (22.60%), beta-sesquiphellandrene (19.61%), alpha-curcumene (10.91%) and the lowest was trans-beta farnesene (0.89%). Based on the screening of red ginger ethanol extract using GC-MS, the most abundant compound is zingiberene with a percentage of 45.99%, this is because zingiberene is a compound that produces a very strong distinctive aroma of ginger [8].

3.2. *Compound Screening*

The results of the identification of compounds from red ginger ethanol extract using GC-MS, then screening using Way2Drug (http://www.pharmaexpert.ru/pass*online*) to find out which compounds have antihypertensive activity by entering the canonical name (SMILES) of the compound used obtained from (https://pubchem.ncbi.nlm.nih.gov). All compounds ranging from zingiberene, farnesene, trans beta-farnesene, alpha-curcumene, and beta-Sesquiphellandrene had antihypertensive activity marked with a value of Pa> 0.3 (data not shown).

3.3. *Docking Experiment*

Docking data such as RMSD, bond energy and inhibition constant (Ki). RMSD is a value that is used to determine whether the prediction of the bond is successful or not by looking at the similarity of the coordinates between the tested ligand and the native ligand. This value is important for the validation of the docking program. Docking is valid if the RMSD value is < 2Å [9]. The greater the RMSD value, the greater the deviation or error in the prediction of the interaction of the ligand with the receptor [10]. The best RMSD value for all docking results (ACE-trans beta farnesene, ACE-alpha curcumene, ACE-zingiberene, ACE-farnesene, ACE-beta sesquiphellandrene) was 0.00.

The value of binding energy (bond energy) used per compound with the lowest value because the lowest value of the binding energy indicated strong the bond formed. Then the lower the value of the inhibition constant indicates the stronger the ligand binds to the receptor. The lowest value of binding energy was selected from every 100x run on each docked compound with sequential results for ACE-trans beta farnesene, ACE-alpha curcumene, ACE-zingiberene, ACE-farnesene, ACE-beta sesquiphellandrene are -5.14 kcal/mol (Ki: 171.89 M), -5.61 kcal/mol (Ki: 76.88 M), -6.20 kcal/mol (Ki: 28.51 M), -5.66 kcal/mol (Ki: 70.84 M), -6.55 kcal/mol (Ki: 15.85 M). Based on these results, the lowest bond energy among the 5 compounds was -6.55 kcal/mol at ACE-beta sesquiphellandrene docking.

Table 2. Results of docking between receptors and ligands

Ligand	RMSD (Å)	*Binding energy* (kcal/mol)	Ki (µM)	Bond Type	Residue
Trans beta-farnesene	0.00	-5.14	171.89	Van der Waals bonds, hydrophobic bonds (Pi-Alkyl and Alkyl)	LEU122, ARG124, ALA89, LEU132, TYR62, ILE88, ALA125, ASN85, TRP59, THR92, GLU123
Alpha-curcumene	0.00	-5.61	76.88	Van der Waals bonds, hydrophobic bonds (Pi-Alkyl and Alkyl)	TRP59, ALA125, ILE88, ASN85, ASN136, ALA89, LEU132, ARG124, GLU123, LEU122, THR92
Zingiberene	0.00	-6.20	28.51	Van der Waals bonds, hydrophobic bonds (Alkyl)	ALA126, LEU127, ALA89, ALA125, LEU132, MET86, ASN90, LEU93, ALA129, PRO128.
Farnesene	0.00	-5.66	70.84	Van der Waals bonds, hydrophobic bonds (Pi-Sigma, Pi-Alkyl and Alkyl)	TYR360, TRP59, HIS91, TYR51, ALA400, LYS118, VAL119, ASP121, THR92, ILE88, LEU122, GLU123
Beta-sesquiphellandrene	0.00	-6.55	15.85	Van der Waals bonds, hydrophobic bonds (Alkyl)	LEU127, ALA125, ALA89, LEU132, MET86, ASN90, LEU93, ALA129, PRO128, ALA126

Based on the two parameters (RMSD and bond energy), these indicated that the beta-sesquiphellandrene include criteria as a good ACE-inhibitor [11] because it interacts with the active site of ACE with various interactions formed such as hydrophobic and hydrophobic bonds. van der Waals bonds [12]. Hydrophobic bonds are residue interactions of non-polar amino acids. This hydrophobic interaction analysis proves that all compounds screened by GC-MS occupy the active binding site receptor (ACE). Meanwhile, Van der Waals bonds are formed due to attractive forces between molecules or atoms that are not charged and are located close together, causing polarization of molecules or atoms [13]. According to Prasetyawati et al. (2021) drug compounds generally interact with receptors by forming weak reversible bonds such as Van Der Waals bonds [9].

The interpretation of the docking results was carried out using PyMOL and the Biovia Discovery Studio Visualizer for knowing interaction between residues involved and the binding that occurred between the ligand and the receptor. The best score of docking result for the ligand compounds that binded with protein target will be used as the basis for determining the compound with the best activity [10].

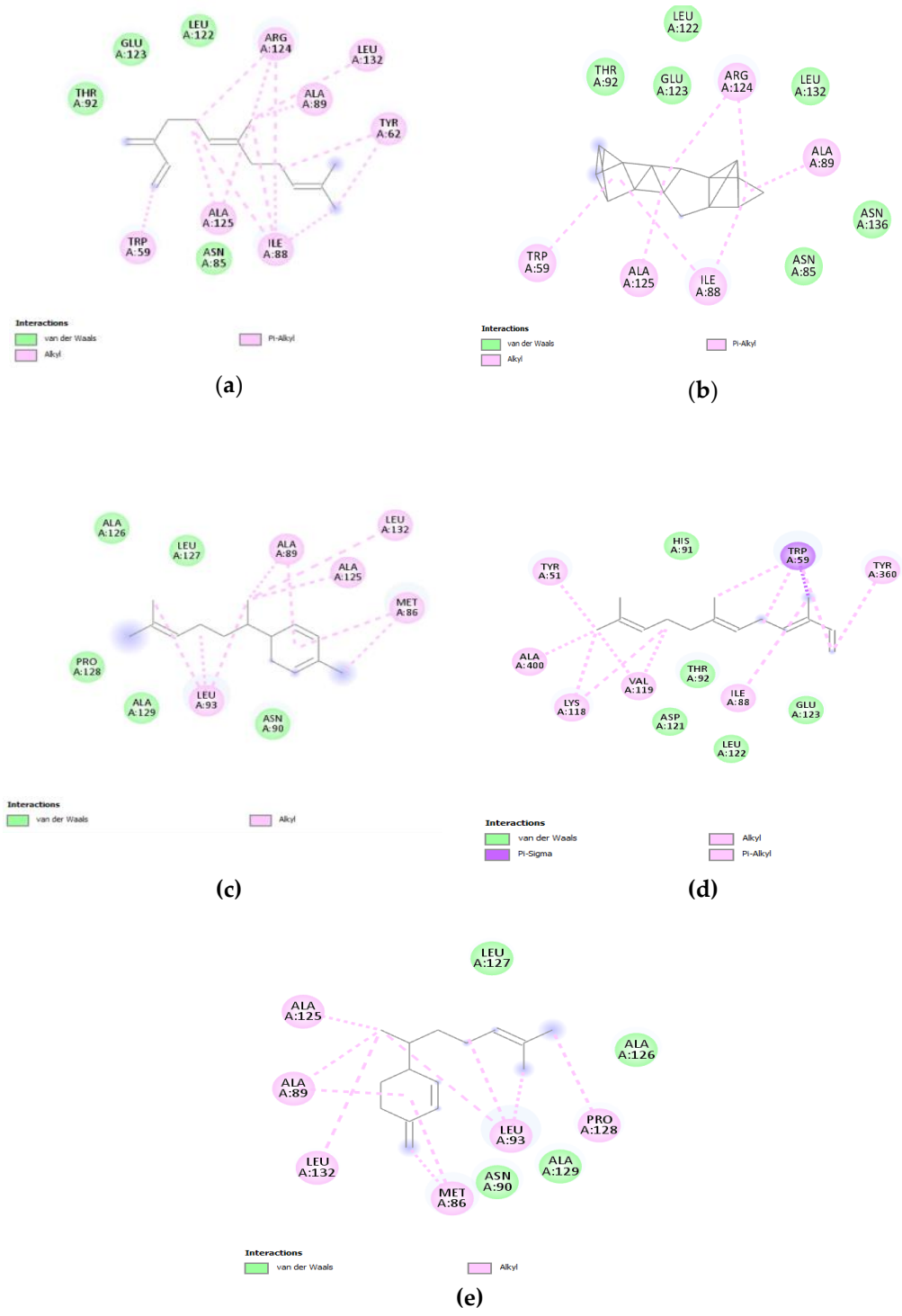

Figure 1. 2D structure of docking result: (**a**) ACE- trans beta farnesene; (**b**) ACE- alpha cuecumene; **(C)** ACE- zingiberene; **(d)** ACE-farnesene; **(e)** ACE- sesquiphelandrene.

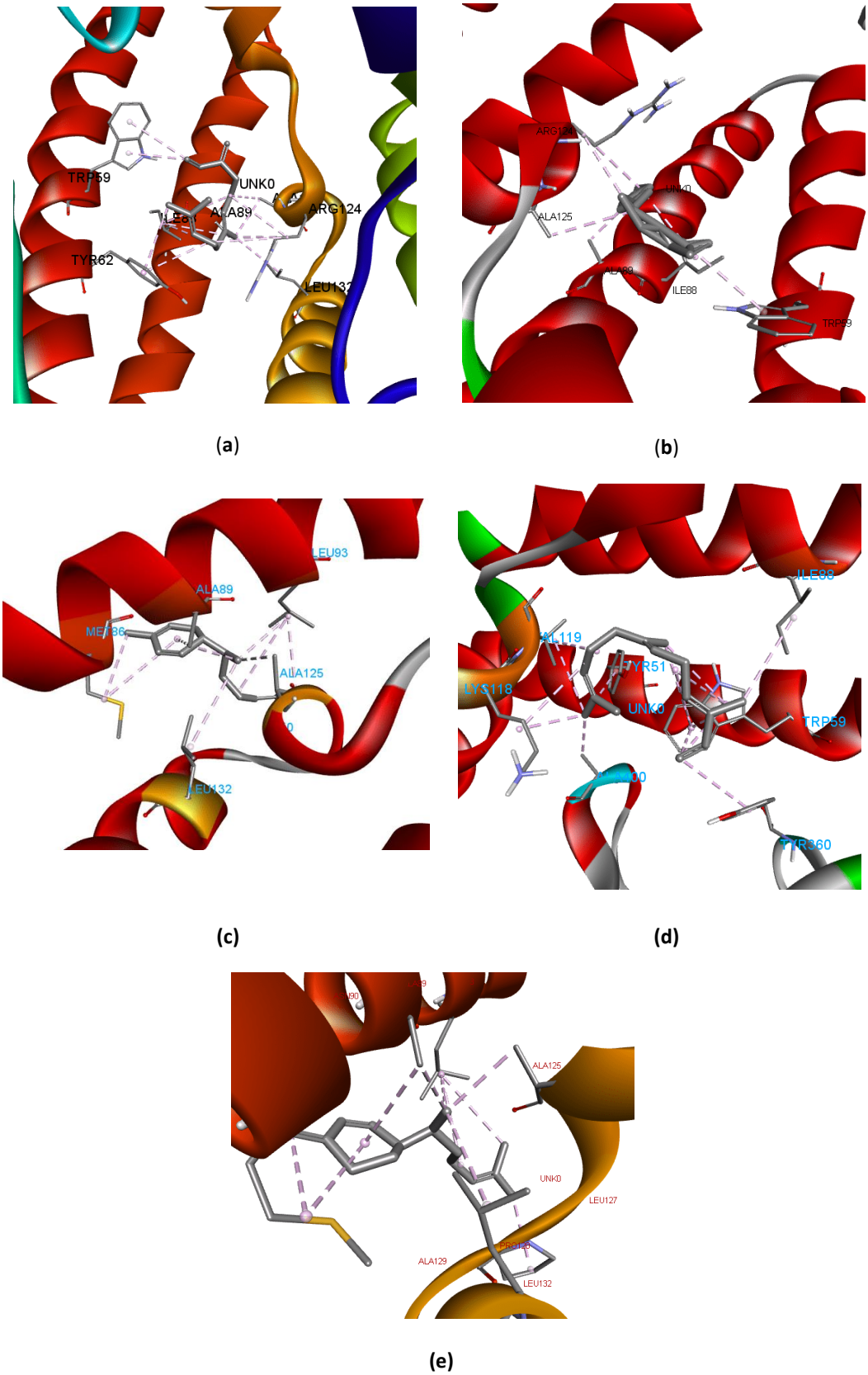

Figure 2. 3D structure of docking result: (**a**) ACE- trans beta farnesene; (**b**) ACE- alpha cuecumene; **(C)** ACE- zingiberene; **(d)** ACE-farnesene; **(e)** ACE- sesquiphelandrene.

4. CONCLUSION

Based on these results, the lowest bond energy among the 5 compounds was -6.55 kcal/mol at docking *ACE*-beta sesquiphellandrene, so the red ginger ethanol extract can be proposed and tested further by in-vitro and in-vivo method as a clinical candidate for antihypertensive drugs.

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Conflicts of interest: The authors declare no conflict of interest

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