JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA), Vol. 6, No. 3, 2021 Hal. 292-303
Chemistry Education Study Program, Universitas Sebelas Maret ISSN 2503-4146
https://jurnal.uns.ac.id/jkpk ISSN 2503-4154 (online)

ANTIOXIDANT POTENCY OF CASSUMUNIN A-C COMPOUNDS FROM BANGLE RHIZOME (*Zingiber cassumunar*) BY MOLECULAR DOCKING ON HUMAN ROS-1 KINASE RECEPTORS

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Received: June 15, 2021 Accepted: December 10, 2021 Online Published: December 23, 2021
DOI : 10.20961/jkpk.v6i3.51995

ABSTRACT

Antioxidants play an role in human health by fighting oxidative stress and preventing chronic disease. Nowadays, molecular docking is used Bangle rhizome (*Zingiber cassumunar*) has a derivative of curcuminoid, cassumunin A, cassumunin B, and cassumunin C. This study was designed to determine the value of binding affinities between cassumunins ligands on human ROS1 kinase receptors, related to their antioxidant activity with ascorbic acid and tocopherol. One set of computational programs is Autodock Tools, Biovia Discovery Studio, and Command Prompt has prepared. These docking results presented the binding affinity values of cassumunin A, cassumunin B, cassumunin C, ascorbic acid, and tocopherol were -9.4 kcal/mol, -9.7 kcal/mol, -9.0 kcal/mol, -5.2 kcal/mol, and -8.1 kcal/mol respectively. RMSD value for the five ligands was ≤ 2Å, showed the validity of the docking results. Cassumunin A-C compound higher affinity compared to ascorbic acid and tocopherol. Based on this computational study, cassumunin A-C the potential compounds to be developed as potent antioxidant agents from natural resources.

Key words: *antioxidant, Bangle rhizome, cassumunin, ROS receptor, molecular docking*

INTRODUCTION

Currently, there are many diseases related to cell damage caused by oxidative stress and free radical substances [1,2,3]. In addition, oxidative stress is associated with the development of several metabolic, chronic disorders or cancers [4,5,6]. Dietary antioxidants play an important role in human health by counteracting oxidative stress and preventing chronic diseases [7]. The importance of antioxidants for maintaining the physiological functions of the liver, kidney, digestive system, and the prevention of cardiovascular diseases and cancer has also been highlighted [8]. Proto-oncogene tyrosine-protein kinase ROS (ROS1), an orphan receptor tyrosine kinase, has been demonstrated to be a potential therapeutic target since crizotinib was approved by the US FDA for the treatment of advanced ROS1-

positive NSCLC [9]. ROS1 plays a crucial role in some of the cellular processes like apoptosis, survival, cell migration, and transformation in different malignancies including colorectal cancer, inflammatory myofibroblast tumor, ovarian cancer, and non-small cell lung cancer. Hence ROS1 becomes a potential drug target [10].

Plants known as precious source with many phytochemical components its potential helps to cure various diseases [11]. Bangle (*Zingiber cassumunar* Roxb.) is a family of Zingiberaceae which is mostly found in Southeast Asia, especially in Indonesia. The part of the plant that was used is the rhizome. Bangle rhizome has been used by the Indonesian people for the treatment of several diseases [12]. Previous research on *Z. cassumunar rhizome*, which is a synonym for *Z. purpureum*, reported the isolation of various types of phenyl butenoids, curcuminoids, and terpenoids [13].

From the rhizomes of *Z. cassumunar*, new complex curcuminoids, namely cassumunins A, B, and C, were isolated by the guidance of both antioxidant and anti-inflammation assays [14]. Indeed, their activity were also shown to be stronger than curcumin. Cassumunins are new complex curcuminoids, and their structures were determined to be 5'-phenylbutenylated curcumins [15]. The structure of the three compounds was presented in Figure 1. The three molecules were used as the ligands for docking with antioxidant receptors, namely human ROS1 kinase (Protein Data Bank code: 3ZBF).

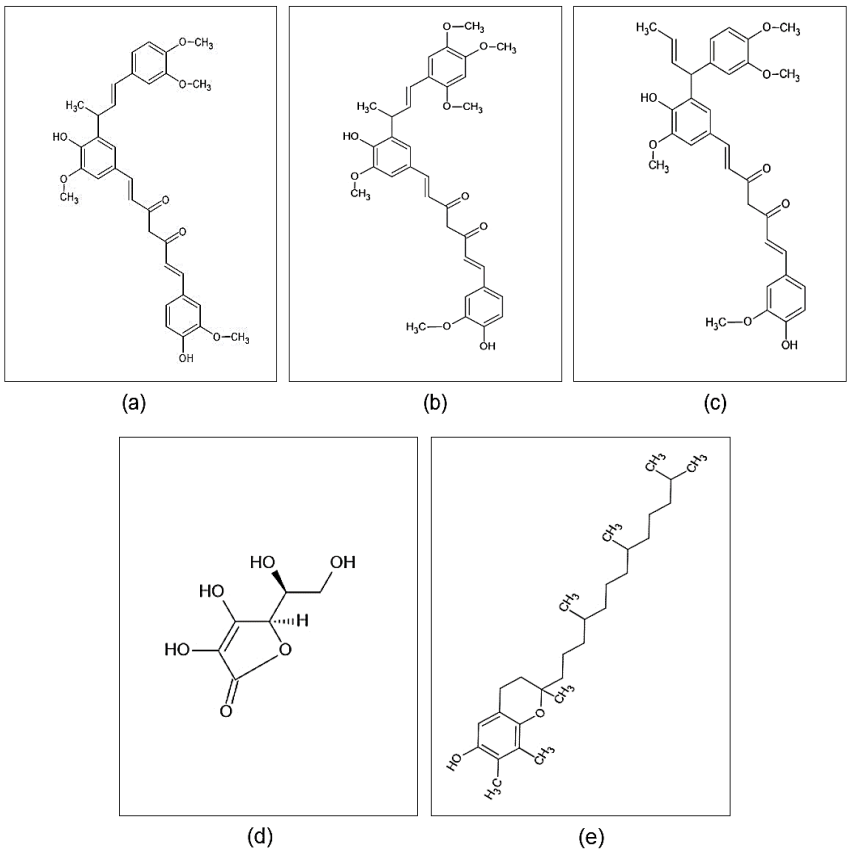


Figure 1. The structure of (a) cassumunin A, (b) cassumunin B, (c) cassumunin C, (d) ascorbic acid, and (e) tocopherol

Molecular docking is a computational simulation used to predict the interaction between a drug/ligand and a receptor/protein by attaching a small molecule (ligand) to the active site of the receptor. This method has been widely used in the process of discovering and developing new drugs with good activity [16,17,18].

This computational method is used in predicting the binding of a drug candidate molecule to its protein target, determining the affinities and activity of a drug candidate molecule, and exploring the three-dimensional geometry of the compound bound to the active site of the protein. Therefore, docking is very important in terms of rational drug design [19, 20, 21].

Furthermore, in this study, molecular docking was done by Autodock software. The docking process used the Vina program, and the ligand and receptor preparation using the Discovery Studio Visualizer program as well as visualizing of docking receptors-ligands result. Autodock-Vina and Discovery Studio Visualizer programs were chosen due to it's free, easy to operate, accurate, have a low error rate, and the reliable results [22]. The Vina method is one of the methods included in the Autodock program. When compared to other free programs, Vina has the advantage of fast and accurate docking.

Based on According to the previous backgrounds, the cassumunin A, cassumunin B, and cassumunin C compounds were testedt, this study tested the antioxidant activity of , and through a molecular docking approach to human ROS-1 kinase receptors, compared to ascorbic acid and tocopherol as a well-known antioxidant. The docking results have been analyzing by the binding affinity and RMSD (Root Mean Square Deviation) value.

METHODS

The hardware used is complete with an Intel Core i3-3770 processor with a CPU speed of 3.40 GHz 8 cores, 1920x1080p monitor resolution, 4GB RAM, NVIDIA GeForce GTX 750 VGA, Windows 10 64-bit. The program used is AutoDock Tools, Biovia Discovery Studio Visualizer, and Command Prompt.

The macromolecules of the ROS receptor were downloaded from the Protein Data Bank and were performed in PDB format. 3D structure of the human ROS1 kinase receptor (PDB code: 3ZBF) as the target receptor. The 3D ligand structure as active compounds from Bangle rhizome was used is cassumunin A, cassumunin B, cassumunin C. The ascorbic acid and tocopherol were used for comparative ligands.

1. Preparation of the ligand

The ligands or drugs were prepared in advance with the Biovia Discovery Studio program. The ligand file format was changed to "pdbqt" which has been optimized by adding hydrogen atoms and changing the active bond to non-rotating using the Autodock Tools program. Data was stored on the drive C of windows.

2. Preparation of the receptor

Receptors were prepared using the Biovia Discovery Studio program to remove protein bonds from the ligands in them and dumped water molecules which were then stored in PDB extension. Further prepara-

tions were made using Autodock Tools to change the file format to "pdbqt" and to add hydrogen bonds which were then combined and tied with the ligands to be prepared for making grid boxes.

3. Determination of grid box

Determination of the grid box is done by determining the coordinates of the active site on the ROS receptor using the Autodock Tools program. It should be noted in the grid options, that all molecules must be entered into the box to obtain valid docking results. The data obtained from the grid box making are spacing in angstrom units, size (x, y, and z), and the value of the center grid box (x, y, and z). The data was stored in a config.txt file to form the basis for docking using Vina and Command prompt.

4. Molecular docking process by Autodock Vina

The ligands and proteins in the "pdbqt" format were copied into the Vina folder, typed the Vina config file into notepad, and saved as a config file. The config file contains data related to the name of the receptor, ligand, box size, and a center box. The process of docking molecules with Vina is executed through the Command prompt with the appropriate parameters.

Molecular docking was started at the Command prompt in a folder containing the receptor, ligand, and config files. The molecular docking process was carried out in the Vina program. After the docking was completed, the binding affinity (kcal/mol) value will be obtained along with the RMSD (Root Mean Square Deviation) value.

5. Analysis of molecular docking results

The docking result analysis included the binding affinity of several docking result modes which were obtained as Log.txt file. The next analysis was ~~is~~ to be looked at the RMSD value to ensure the validity of docking result data. If the RMSD value <2 Å indicated the validity of the docking result [23].

6. Visualization of docking results

The visualization results of interaction between receptors and ligands were performed by Discovery Studio Visualizer program. Visualization was carried out by looking at the amino acid residues produced in a 2D model.

RESULTS AND DISCUSSION

A macromolecule involved in the defense system against free radicals has been targeted for molecular docking. One of the antioxidant receptors, the human ROS1 kinase, belongs to the category of protein kinases that perform very similar functions in humans [24]. These findings suggest that MAPK (Mitogen-Activated Protein Kinase) is not only activated by ROS (Reactive Oxygen Species) but also regulates ROS levels in eukaryotic cells [25-26]. The antioxidant activity of cassumunin A-C was shown by the inhibitory activity of linoleic acid autoxidation [27].

The first step of this study was preparing the ligands and protein. All the ligands and macromolecular proteins that have been downloaded were prepared using the Autodock Tools program to add hydrogen bonds and change the active bonds to non-rotating bonds. The macromolecules were

prepared to obtain protein without native ligands and water with Discovery Studio software. The ligand file is saved in pdbqt format. The results of preparation cassumunin A-C, ascorbic acid, and tocopherol were presented in Figure 2.

Protein files were prepared using the Autodock Tools program to add polar hydrogen bonds, then a non-polar bond was carried out. The protein was bound with the ligands and inserted into a grid box. Validation using a grid box between ligands and proteins was performed on each docking algorithm.

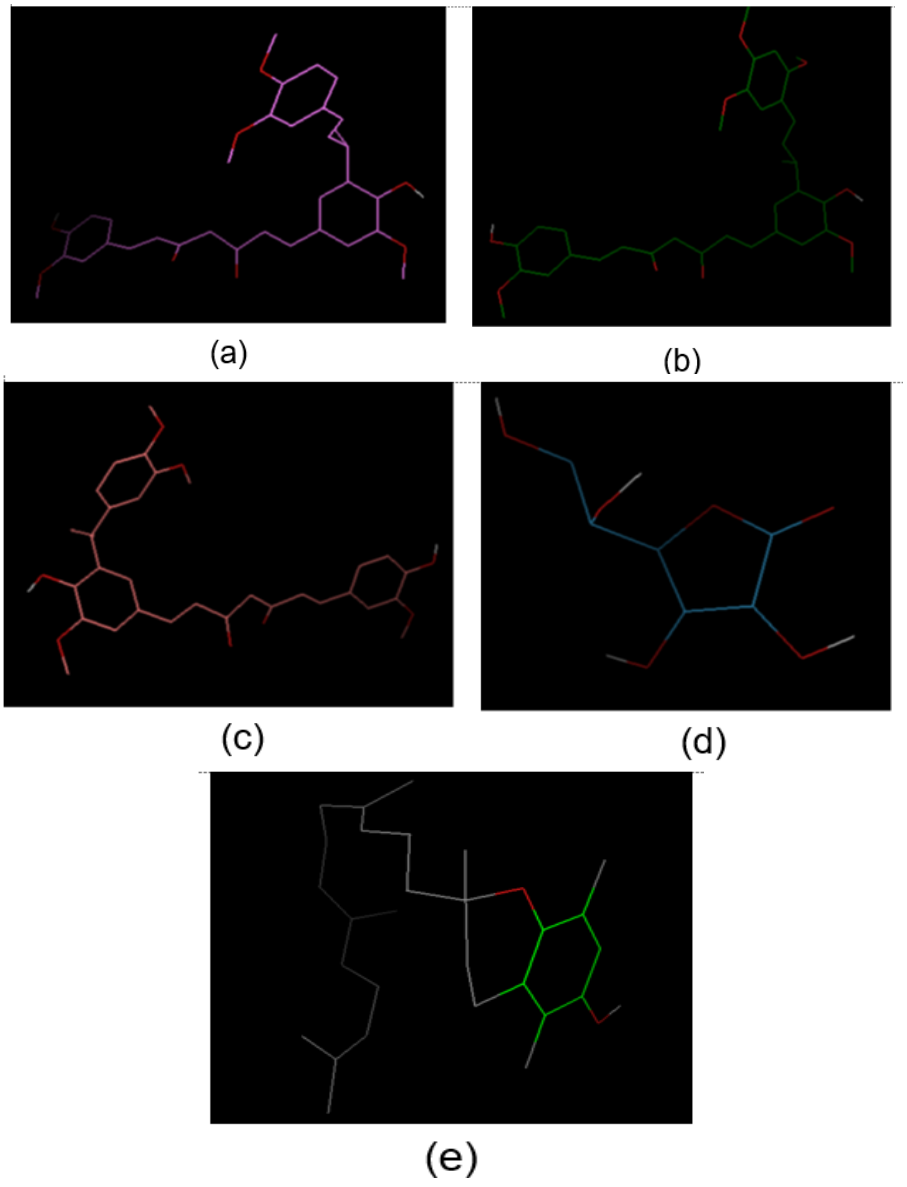


Figure 2. Results of the ligand preparation (a) cassumunin A, (b) cassumunin B, (c) cassumunin C, (d) ascorbic acid, and (e) tocopherol in pdbqt file format

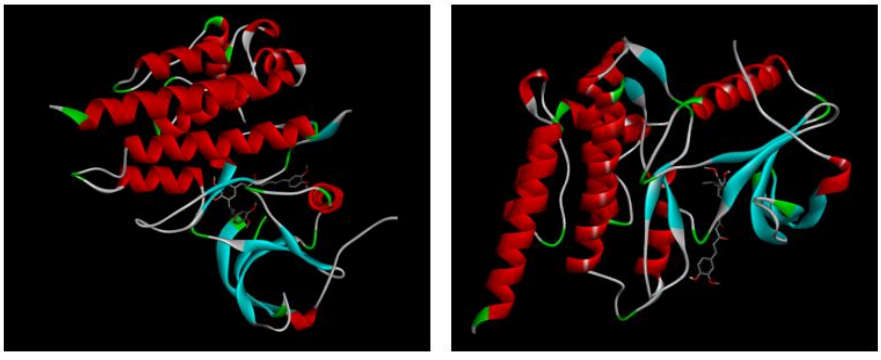
The grid box was done by determining the central coordinate value and the grid box magnitude where ligands and proteins interaction. The docking process was run through the Command Prompt program by first preparing the file of pdbqt ligand, pdbqt

receptor, and config.txt in one folder along with a copy of the vina and vina split files. The docking interactions between the ligands and human ROS1 kinase receptors, and also the binding affinity and RMSD values were performed in Table 1.

Table 1. The molecular docking result of a ligand with human ROS1 kinase receptors

Ligand	Binding affinities (kcal/mol)	RMSD	Amino acid bonds
Cassumunin A	-9.4	0.000	GLU A:1990, THR A:1987, GLN A:1989, ARG A:2083, ASP A:2033, VAL A:1959, LEU A:2086, GLU A:2027, LEU A:2026, ALA A:1978, LEU A:2010, LYS A:1980, ASP A:2102, SER A:1953, GLU A:1993, GLY A:1952
Cassumunin B	-9.7	0.000	GLU A:2027, LEU A:2026, LEU A:2086, VAL A:1959, LEU A:2010, ALA A:1978, MET A:2029, LEU A:1951, GLY A:2032, ARG A:2083, ASP A:2033, THR A:2036, LYS A:2040, LYS A:2090, ASP A:2091, TYR A:2092, GLY A:1952, SER A:1953, ASP A:2102
Cassumunin C	-9.0	0.000	LYS A:2003, GLU A:1935, PHE A:2075, SER A:2002, LYS A:1996, HIS A:1999, LEU A:2000, ALA A:2106, ARG A:2107, ASP A:2108, TYR A:2110
Ascorbic Acid	-5.2	0.000	LEU A:2056, LEU A:2053, PRO A:2187, ASN A:2185, CYS A:2186, PRO A:2183, LEU A:2155, ARG A:2184, HIS A:2159, THR A:2156, LEU A:2157, ARG A:2042
Tocopherol	-8.1	0.000	ASP A:2108, LEU A:2000, LYS A:1996, LYS A:2003, HIS A:1999, PHE A:2075, GLU A:1935

A total of five ligands were incorporated into the docking experiments including cassumunin A, cassumunin B, cassumunin C, ascorbic acid, and tocopherol. The bond energy formed was taken by the most negative value because it has a strong interaction. Binding affinity is a measure of a drug's ability to binding receptors. The smaller binding affinity value is the higher affinity between the receptor and the ligand, and the greater binding affinity value is the lower affinity between ligand and receptors [28]. The binding affinity value based on Table 1 showed the best bond affinity of the cassumunin B, cassumunin A, cassumunin C, ascorbic acid, and tocopherol compounds respectively, -9.7 kcal/mol, -9.4 kcal/mol, -9.0 kcal/mol, -5.2 kcal/mol, and -8.1 kcal/mol.



(a) (b)

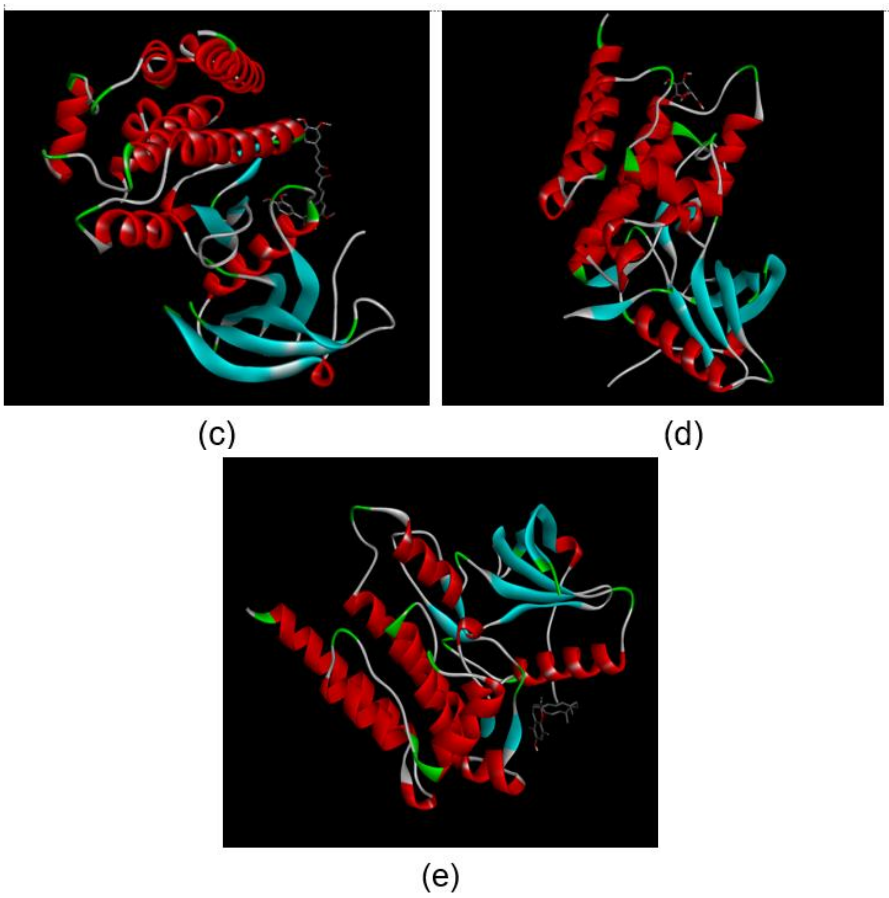


Figure 3. Results in a 3D model of the docking molecular interactions between ligands (a) cassumunin A, (b) cassumunin B, (c) cassumunin C, (d) ascorbic acid, and (e) tocopherol on human ROS1 kinase receptor

Based on the 3D model of docking interaction in Figure 3, the docked complex of each ligand interacting with the binding site of human ROS1 kinase receptors in some specified amino acids. The cassumunin B test ligand predicted to have a better affinity for the active site on ROS1-kinase receptor, based on the lower energy and more hydrogen bond interaction. Therefore, this finding is indicated that curcuminoids in Bangle, especially cassumunin A, cassumunin B, and cassumunin C have the potential activity to protect against ROS and can be promoting as a lead compound.

In addition, regarding the validity test of the docking result, the RMSD value was used as the validity parameter. The ligand shape with the smallest RMSD is used as a representation of the form of interaction between the test ligands with receptors [28]. Results of the validation method showed that the RMSD value was ≤ 2Å (Table 1). This value shows the docking calculation between receptor and ligand gives almost the same result because it has a value of ≤ 2Å [29] proving this docking has been valid. The 2D Visualization model of the docking results for cassumunin A, cassumunin B, cassumunin C, ascorbic acid, and tocopherol ligands was present in Figure 4.

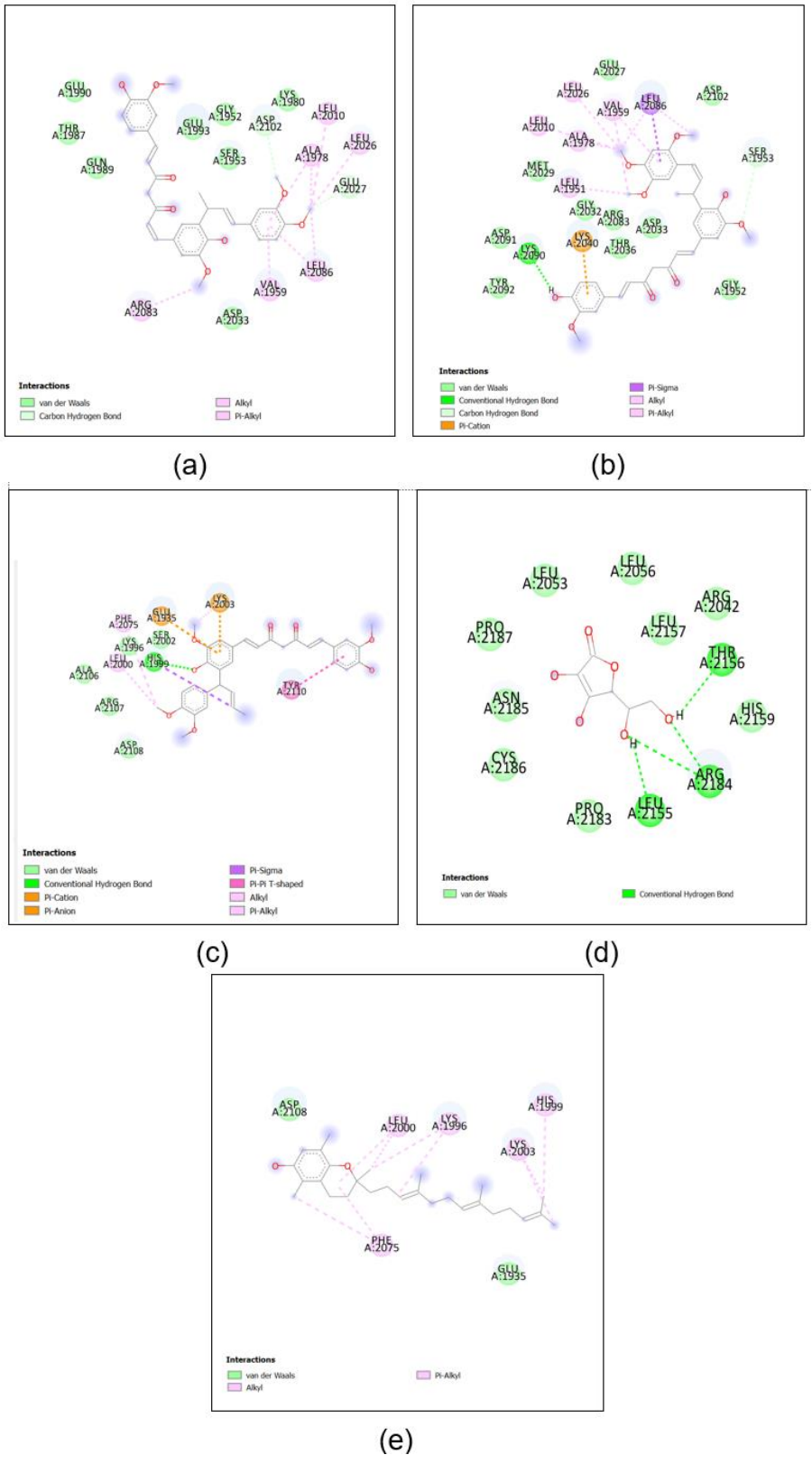


Figure 4. Visualization of the docking results in a 2D model for the ligands (a) cassumunin A, (b) cassumunin B, (c) cassumunin C, (d) ascorbic acid, and (e) tocopherol

Furthermore, as shown in Figure 4 the cassumunin B has the most amino acid bonds compared to the other test ligands. The interactions formed from the cassumunin B ligand complex with the human ROS1 kinase receptor consist of one hydrogen bond, 10 Van der Waals bonds, six hydrophobic bonds, and one electrostatic interaction. This result was reinforced the more effective binding of cassumunin B on the human ROS1 kinase receptor and has the most amino acid residue, correlated with its affinity value. In the comparison with the ascorbic acid and tocopherol ligands, both ligands were only bind with some amino acids in less than three type interactions. ascorbic acid ligand revealed two type interaction, namely van der waals and hydrogen bond. Whether Tocopherol ligand showed two type interaction that supported by van der waals and hydrophobic bond. Therefore the binding affinity of ascorbic acid and tocopherol were lower than cassumunins that only -5.2 kcal/mol for ascorbic acid and -8.1 kcal/mol for tocopherol.

In line with the molecular docking result, Zingiber cassumunar has shown main pharmacological activities which include analgesics, antimicrobials, anti-inflammatory, anticancer, and radical scavenging activities, antimalarial, and other activities in both in vitro, in vivo and also clinical evaluation [[12]..

The target protein tested was human ROS kinase, where ROS1 is one of 58 receptor tyrosine kinases, and one of two orphan receptor tyrosine kinases where its ligand is unknown. ROS1 is evolutionarily related to ALK. ROS1 rearrangement was discovered in glioblastoma in 1987, in non-small-cell lung cancer (NSCLC) in 2007, and in cholangiocarcinoma in 2011 [30], therefore the receptors can also be targeted for the treatment of certain types of cancer.

In the term of antioxidant activity, Bangle rhizome hs shown antioxidant effect. The administration of ethyl acetate fraction of Bangle extract showed the increasement of IL- 10 and IL-14 expression [31]. Zingiber cassumunar rhizome has effectively increased antioxidant activity and minimized the adverse effects of high-fat diet by increaseasing of SOD enzyme activity [32].

Afterwards, as part of cassumunins development as the lead compound in antioxidant activity, the molecular docking study of cassumunins analog will be investigated.

CONCLUSION

In the conclusion, based on the molecular docking results, the three active compounds of the curcuminoid group from Bangle rhizomes, namely cassumunin A, cassumunin B, and cassumunin C were predicted have better antioxidant activity compared to ascorbic acid and tocopherol. The Cssumunins presented the higher binding affinity value on human ROS1 kinase receptor. Cassumunin B compound has the best affinity value, -9.7 kcal/mol. This study was also supported the development of Cassumunins as the potential antioxidant compounds from natural resources.

ACKNOWLEDGEMENT

This research is supported by the Ministry of Higher Education in *Hibah Penelitian Dasar Unggulan Perguruan Tinggi* 2021.

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