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An In Silico Study Anti-Inflammatory of Active Compound of Ramania Leaves Extract (Bouea macrophylla Griffith) Against Angiopoietin-2

Irham Taufiqurrahman[1*], Isyana Erlita[2], Mira Hadistina[3], Juliyatin Putri Utami[4], Andi Zulkifli[5], Veni Hadju[5], Eko Suhartono[6], Muhammad Irpan Hendrawan[7]

Departement of Oral and Maxillofacial Surgery, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin – Indonesia[1]
Department of Conservative Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia[2]
Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin – Indonesia[3]
Department of Biomedicine Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia[4]
Faculty of Public Health, Hasanuddin University, Makassar – Indonesia[5]
Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Indonesia[6]
Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia[7]

Corresponding Author: 1*

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ABSTRACT

Traumatic Dental Injury (TDI) is a trauma that occurs when the hard and soft tissues of the oral cavity receive an impact. The prevalence of traumatic dental injury (TDI) experienced at all ages touched the percentage of 20%. According to Petti and colleagues, the prevalence of TDI in primary teeth is higher than in permanent teeth. The prevalence of TDI worldwide by bibliometric analysis in 1999-2018 touched the percentage of 6%-59%. To determine the active compound binding of Bouea macrophylla G. (ramania) as an anti-inflammatory by inhibiting the protein angiopoietin-2 (Ang-2) in silico. Experimental design through in silico method. Methyl ricinoleate compound has RMSD (Root Mean Square Deviation) value of 0 and negative binding affinity value, hydrogen bonding, and the same residue as the comparison ligand, atorvastatin. All active compounds of Bouea macrophylla G. has passed the drug likeness test with Lipinski's Rule parameters. Based on the results of the ADMET test, it is known that the absorption of methyl ricinoelate compounds can be absor bed well, the absorption of Caco-2 cells is the highest compared to the 9 active compounds of Bouea macrophylla G. and atorvastatin, the volume of distribution and total clearance is better than atorvastatin, does not inhibit CYP50, is notmutagenic, carcinogenic, and not hepatotoxic, has lower Oral Rate AcuteToxicity (LD50) and Oral Rate Chronic Toxicity (LOAEL) values compared to atorvastatin. Methyl ricinoloeate can potentially be an anti- inflammatory drug through the inhibition of Ang-2.

1. Introduction

Traumatic Dental Injury (TDI) is a trauma that occurs when the hard and soft tissues of the oral cavity receive an impact [1]. The prevalence of traumatic dental injury (TDI) experienced at all ages touched the percentage of 20%. According to Petti et al (2018), the prevalence of TDI in primary teeth is higher than in permanent teeth. The prevalence of TDI worldwide by bibliometric analysis in 1999-2018 touched the percentage of 6%-59% [2], [3]. TDI trauma that occurs often involves many tissues and takes a lot of timeto treat, so treatment is relatively expensive and difficult. Some drugs such as antibiotics are also used in addition to surgery [1], [4].

The use of drugs in wound healing is available with various kinds and types, including the use of herbal medicines. Herbal medicine is an alternative in medicine because the price is cheap, the ingredients are relatively easy to obtain and do not cause side effects. Bouea macrophylla G. can be selected as a plant that can play a role in accelerating wound healing. It is known that the leaves of Bouea macrophylla G. contain compounds of caryophyllene, humulene; hexadecanoic acid, ethyl ester (palmitic acid); phytol; squalene; vitamin E; retinol, acetate; and y-himachalene which has potential as an anti-inflammatory [5- 7].

Treatment of wound healing in soft tissue aims to clean the wound area from foreign objects, dead cells and bacteria so as to support the healing process. As the body's response to wound healing, there is an inflammatory process that involves: a number of endothelial growth factors. One of the proteins derived from endothelial growth factor is the protein angiopoietin-2 (Ang-2). Ang-2 is the second member of the angiopoietin family that plays a role in blood formation and is known as a protein ligand and is secreted from the receptor tyrosine kinase (Tie2). Ang-2 acts as a vascular disruptor which is its role as a natural Tie2 antagonist by counteracting the vascular-stabilizing activity of the Tie2 receptor while simultaneously blocking its interaction with its canonical agonist ligand, Ang-1 during inflammation. Thus, when the Ang-2/Tie2 signaling pathway is inhibited, vessel-in-vessel growth can be markedly enhanced in inflammation and new fields can be discovered for therapeutic exploration [6], [8- 10].

The comparison ligand used was an Ang-2 inhibitor, namely atorvastatin. The comparison protein used in protein-to-protein docking is Tyrosine Kinase-2 (Tie2). This study predicts the therapeutic target for Ang-2 by using 10 active compounds Bouea macrophylla G.

2. Materials & method

2.1 Ligand and Protein Preparation

Receptors namely Ang-2 and Tie2 were prepared using Biovia Discovery Studio version 21 application to remove water molecules and residues. The ligands, namely the active compound Bouea macrophylla G. and atorvastatin, were prepared using the Open Babel program from PyRx version 0.8 to minimize energy, optimize, and convert the format to .pdb.

2.2 Validation

The validation process was carried out by re-docking the Ang-2 protein with its natural ligand (calcium ion) (CID:336285734). The parameter used is the RMSD (Root Mean Square Deviation) value. The validation results obtained are 0. RMSD is a value associated with the deviation of the binding position. The docking

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method is said to be valid if the RMSD value is 2, so the docking method can be used for the docking test of activecompounds.

2.3 Protein-Ligand Docking and Visualization

Molecular docking simulation using AutoDock Vina program which is integrated with PyRx version 0.8. The analysis and visual prediction of the protein-ligand complex virtual from the docking stage were analyzed and visualized using the Biovia Discovery Studio version 21 application. The interaction side was analyzed based on the residual ligand interaction and structural conformation. Ang-2 was also docked with atorvastatin to compare the binding affinity values of 10 Bouea macrophylla G compounds.

2.4 Prediction of drug-likeness, pharmacokinetics, and safety

Lipinski's Rule of 5 used to predict drug similarity to the active compound, which was evaluated by the pkCSM website (http://biosig.unimelb.edu.au/pkcsm/prediction) and PubChem (https://pubchem.ncbi.nlm.nih.gov/).

3. Results

In Table 1. it can be seen that the highest molecular weight is owned by atorvastatin with a weight of 558.65 g/mol and the lowest weight is a compound of humulene and a compound of *γ-himachalene* with a weight of 204.357 g/mol. The best logP values were owned by *carophyllene* compounds and *γ-himachalene* compounds with a logP of 4.7252. While the largest is squalene compound with logP 10,605. All of the active compounds have good hydrogen acceptors, Bouea macrophylla G. Meanwhile, all of the tested active compounds of Bouea macrophylla G. have < 5. The widest topological polar surface area (TPSA) (Table 1) is owned by atorvastatin compounds with an area of 112. Based on Table 1. it was found that all active compounds of Bouea macrophylla G. leaf extract passed the Lipinski's rules test.

All the active compounds of Bouea macrophylla G. tested (Table 2.) were well absorbed in the human intestine. The highest absorption of Caco-2 cells was methyl ricinoleate with a value of 1.57 log cm/s. The highest VDss value was found in vitamin E compounds with a VDss value of 0.873 log L/kg.

The 10 active compounds tested Bouea macrophylla G. (Table 2.) did not act as CYP2D6 inhibitors, so their pharmacokinetic properties were enzymatic. The highest total clearance value was owned by *squalane* compound with a value of 1.791 log mL/minute/kg.

All tested Bouea macrophylla G. compounds were neither mutagenic nor carcinogenic, nor was atorvastatin. In the hepatotoxicity test (Table 2.), it was found that all Bouea macrophylla G. compounds were not hepatotoxic, except for the retinol compound, acetate, which resulted in hepatotoxicity as well as *atorvastatin*. The highest LD50 is owned by atorvastatin with a value of 2.877 mol/kg. The highest LOAELwas owned by *atorvastatin* with a value of 4.839 mol/kg.

Table 3. The binding affinity value obtained varies from -3.3 kcal/mol to -6.5 kcal/mol. The lowest bond value was owned by the Ang-2_*atorvastatin* bond of -6.5 kcal/mol. While the highest is the Ang2-*palmitic acid* bond. Ang-2_Bond *Methyl ricinoleate* has 2 hydrogen bonds. In addition, the Ang-2_*methyl ricinoleate* bond has the same residue as the Ang-2_*atorvastatin* bond residue, namely the GLU35 residue. The result of Ang-2_pristanal docking also has 2 hydrogen bond interactions. Meanwhile, Ang-2_*retinol*, *acetate*; Ang-2_*phytol*; and Ang-2_*palmitic acid* has a hydrogen bond type interaction, while the Ang-2_γ- *himachlene* bond; Ang-2_vitamin E bond; bond Ang-2_*carophyllene*; bond Ang-2_*humulene*; Ang- 2_*squalene* bonds have no hydrogen-type bond interactions.

The most negative binding affinity value from the results of docking to protein (Table 4.) is the result of docking tyrosine kinase-2 (Tie2) with Ang-2_*carophyllene* bonds with a value of -8.1 kcal/mol, higher than the binding affinity value of Tie2_Ang- 2_atorvastatin with a value of -7.5 kcal/mol. While the highest is Tie2_Ang-2_palmitic acid with a value of -4 kcal/mol.

The amino residues on the interaction bonds found in the protein-to-protein docking results (Table 4.) were almost all similar to the amino acid residues from the docking of Ang-2 with the active compounds Bouea macrophylla G. and *atorvastatin*, except for the docking results of Tie2_Ang-2_*pristanal* at residuesMET857 and PHE869, Tie2_Ang-2_*methy ricinoleate*. Tie2_Ang-2_*methyl ricinoleate* has the same residues with a total of 12 residues that are the same as Tie2_Ang-2_*atorvastatin*, namely at residues LYS855, ARG856, VAL829, LEU839.

Hydrogen bond interactions from protein to protein docking results (Table 4.) from Tie2_Ang-2_10 active compound Bouea macrophylla G. and Tie2_Ang-2_*atorvastatin* bonds were mostly found in Tie2_Ang-2_atorvastatin bonds with 5 hydrogen bonds at residues LYS855, ARG856, LYS858 and bonds Tie2_Ang-2_*methyl ricinoleate* on residues LYS855, ARG856 (3.25349 and 3, 37493), GLN837, and CO. Meanwhile, the Tie2_Ang-2_*carophyllene* bond, the Tie2_Ang-2_*pristanal* bond, the Tie2_Ang-2_*humulene* bond, and the Tie2_Ang-2_γ-*himachlane* bond did not have hydrogen bonds.

Table 1. Results of Physicochemical Properties and Toxicity of Active Compounds Bouea macrophylla G. and Comparative Ligand Atorvastatin Correlation with Lipinski's Rule.

Compound	Molecular Weight (g/mol)	LogP	Hydrogen Acceptors (n)	Hydrogen Donor (n)	TPSA (Topological Polar Surface Area)	Lipinski's Law Deviations	Pass/No
Carophyllene	204.537	4.7252	0	0	0	0	Graduated
2-Methyl-cis-7, 8-epoxynonadecane (Pristanal)	296.539	6.8911	1	0	12.5	1	Graduated
Humulene	204,357	5.0354	0	0	0	1	Graduated
Hexadecanoic acid, ethyl ester (Palmitic acid)	284,484	6.1884	1	1	37.3	1	Graduated
Oxiraneundecanoic acid, 3-penthyl-methyl ester, trans- (Methyl ricinoleate)	312,494	5.5673	2	1	46.5	1	Graduated
Phytol	296.539	6.3641	1	1	20.2	1	Graduated

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Retinol, Acetate	328,496	6.0811	2	0	26.3	1	Graduated
Vitamin E	430.717	8.84026	2	1	29.5	1	Graduated
γ-himachalene	204,357	4.7252	0	0	0	0	Graduated
Squalene	410.73	10,605	0	0	0	1	Graduated
Atorvastatin	558.65	6,3136	5	4	112	2	Graduated

Table 2. Results of Physicochemical Properties and Toxicity of Active Compounds Bouea macrophylla G. and Comparative Ligand Atorvastatin

Compound	Caco-2 permeability (nm. sec-1)	Human Intestinal Absorption (%)	VDss (log L/kg)	CYP 2D6 Inhibitor	Total Clearance	AMES Toxicity	Hepatotoxicity	Oral Rate Acute Toxicity (LD50) (mol/kg)	Oral Rate Chronic Toxicity (LOAEL) (log mg/kg_bw/day)
Carophyllene	1.434	95.304	0.653	*No*	1.088	*No*	*No*	1,678	1.44
2-Methyl-cis-7, 8-epoxynonadecane	1.32	91,567	0.451	*No*	1,631	*No*	*No*	1.382	1.028
Humulene	1.421	94,682	0.505	*No*	1,282	*No*	*No*	1,766	1.336
Hexadecanoic acid, ethyl ester	1.38	92,158	-0.679	*No*	1,678	*No*	*No*	1.46	3.298
Oxiraneundecanoic acid, 3-penthyl-methyl ester, trans-	1.57	91,956	-0.509	*No*	1.615	*No*	*No*	1.573	2,701
Phytol	1.487	89,892	0.416	*No*	1,686	*No*	*No*	1,646	1.024
Retinol, Acetate	1.188	94.33	0.408	*No*	1.503	*No*	***Yes***	1,673	2.276
Vitamin E	1,215	90,045	0.873	*No*	0.801	*No*	*No*	2,243	2.445
γ-himachalene	1.418	94.556	0.648	*No*	1.093	*No*	*No*	1,681	1.346
Squalene	1.193	89,581	0.282	*No*	1,791	*No*	*No*	1995	0.902

Atorvastatin	0.23	59,861	-1.918	*No*	0.437	*No*	***Yes***	2.877	4.839

Table 3. Results of Docking Ang-2 with Bouea macrophylla G. Active Compound and Comparative Ligand Atorvastatin

Compound	Binding Affinity (kcal/mol)	Amino Residue	Category
Carophyllene	-5.3	ILE309, LYS310	*Hydrophobic*
2-Methyl-cis-7, 8-epoxynonadecane (Pristanal)	-4.2	GLY347, GLY345	*hydrogen*
		LYS310, VAL344, ILE309, PRO349, LYS340	*Hydrophobic*
Humulene	-4.8	-	-
Hexadecanoic acid, ethyl ester (Palmitic acid)	-3.3	PHE346	*hydrogen*
		ILE309, VAL344, LYS310	*Hydrophobic*
Oxiraneundecanoic acid, 3-penthyl-methyl ester, trans-(Methyl ricinoleate)	-4.5	GLU395, *GLU358	*hydrogen*
		PRO303	*Hydrophobic*
Phytol	-4	GLU308	*hydrogen*
		LYS310	*Unfavorable*
		PRO349, ILE309, VAL344	*Hydrophobic*
Retinol, Acetate	-5.7	LYS372	*hydrogen*
		ALA494, VAL370, LEU386	*Hydrophobic*
Vitamin E	-5.4	PHE469, PRO452, PHE469, PHE469, TYR475, TYR475	*Hydrophobic*
y-himachalene	-5.8	MET458	*Hydrophobic*
Squalene	-4,4	ILE309, LYS310, PRO349, VAL344 VAL344	*Hydrophobic*
Atorvastatin	-6.5	SER393, TYR391	*hydrogen*
		***GLU358, *GLU358**	*Halogen*
		ASN365, HIS402	*Unfavorable*

* =The same residue as the comparison ligand

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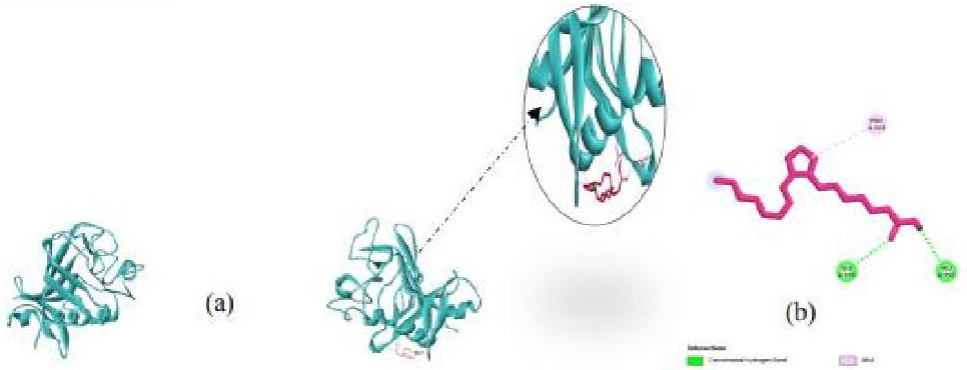

Figure 1.a. 3D Structure of Ang-2 docked with *Oxiraneundecanoic* acid, 3-penthyl-methyl ester, trans- (*methyl ricinoleate*), b. 2D structure of Ang-2 docked with *Oxiraneundecanoic* acid, 3-penthyl-methyl ester, trans- (*methyl ricinoleate*)

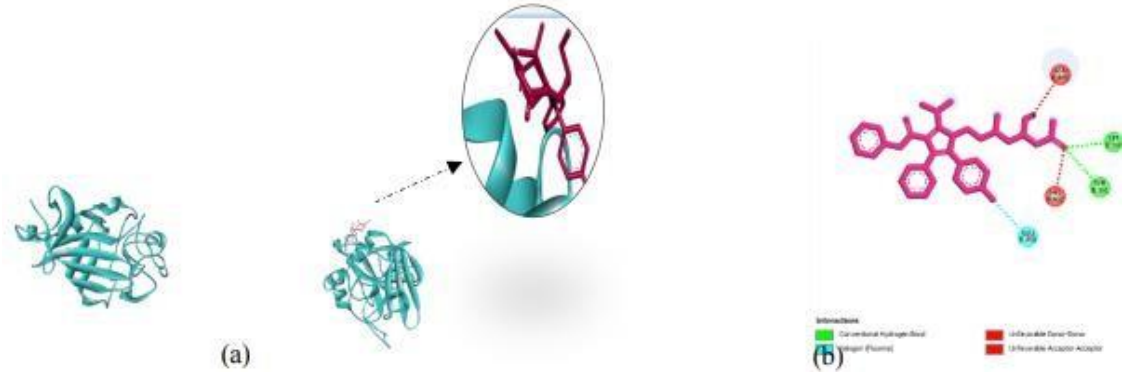

Figure 2.a. 3D structure of Ang-2 docked with Atorvastatin, b. 2D structure of Ang-2 docked with Atorvastatin

Table 4. Results of Docking Tie2_Ang-2 with Bouea macrophylla G. Active Compound and Comparative Ligand Atorvastatin

Compound	Binding Affinity (kcal/mol)	Amino Residue	Category
Carophyllene	**-8.1**	***VAL829**	*Hydrophobic*
2-Methyl-cis-7, 8-epoxynonadecane (Pristanal)	-4.7	***VAL829, *VAL829, *VAL829, *ARG856, *ARG856, *LYS858 *LYS858,** MET857, *LEU839, *LEU839, *ARG856, *VAL829, PHE869	*Hydrophobic*
Humulene	-7.9	**VAL829**	*Hydrophobic*
Hexadecanoic acid, ethyl ester (palmitic acid)	-4	***LYS855, *ARG856**	*hydrogen*
		***VAL829, * VAL829, *ARG856, *ARG856, *LEU839**	*Hydrophobic*
Oxiraneundecanoic acid, 3-penthyl-methyl ester, trans-(Methyl ricinoleate)	-5.6	***LYS855, *ARG856,** GLN837, *ARG856, CO,	*hydrogen*
		***VAL829, *VAL829, *ARG856, *VAL829, *VAL829, *LEU839, *ARG856, *ARG856, *LEU839**	*Hydrophobic*
Phytol	-5	***ARG856**	*hydrogen*

		***VAL829, *VAL829, *LEU839, *LYS858, *LYS858**	*Hydrophobic*
Retinol, Acetate	-6.7	THR1016, ASN1018	*hydrogen*
		TYR1012, TYR992, VAL1014, TYR1012	*Hydrophobic*
Vitamin E	-6.8	***LYS855**	*Hydrogen Bond; Electrostatic*
		***VAL829, *VAL829, *LEU839, *ARG856, *ARG856, *VAL829, *VAL829, *LEU839, *ARG856**	*Hydrophobic*
y-himachalene	-7.1	***VAL829, *LEU839, *ARG856, *VAL829, *VAL829, *LEU839**	*Hydrophobic*
Squalene	-4.8	LYS1059, LEU1061, ALA1114, LYS1059, TYR1068, TYR1068	*Hydrophobic*
Atorvastatin	-7.5	***LYS855, *ARG856, *LYS858, *ARG856**	*Hydrogen Bond*
		***LYS855**	*Hydrogen Bond; Electrostatic*
		***LEU839, *ARG856, *VAL829, *VAL829, *LEU839, *ARG856**	*Hydrophobic*

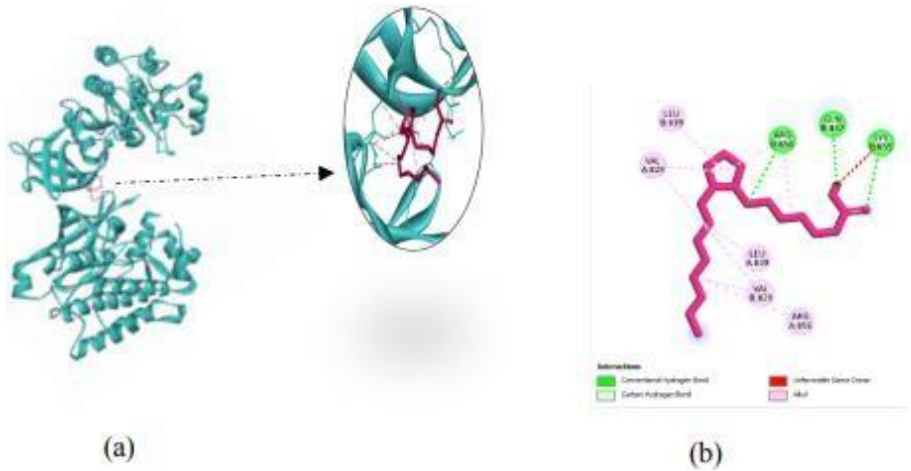

Figure 3.a. Result 3D Structure Docking Protein Tie2_Ang-2 with *Methyl Ricinoleate*, b. 2D Structure of Tie2_Ang-2 Protein Docking Result with *Methyl Ricinoleate*

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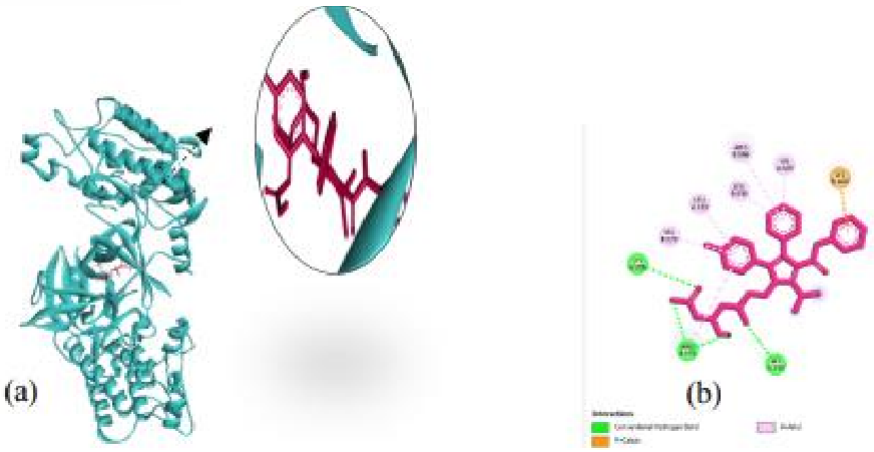

Figure 4. a. Result 3D Structure of Docking Protein Tie2_Ang-2 with Atorvastatin, b. 2D Structure of Tie2_Ang-2 Protein Docking Results with Atorvastatin

4. Discussion

Table 1. All active compounds of Bouea macrophylla G. can be potential candidates for oral drugs because all active compounds of Bouea macrophylla G. leaves passed the Lipinski test. [11] stated that good absorption is absorption that meets 3 or more Lipinski's rules which include: molecular weight not more than 500 g/mol, number of H-bond acceptors not more than 10, Number of H-bond donors not more than 5, and LogP not more than 5 (or MlogP > 5) [12].

In the pharmacokinetic test results (Table 2.), all tested active compounds of Bouea macrophylla G. were well absorbed in the human intestine, because the percentage absorption of all tested compounds was >89%. Based on [13], the value of good absorption in the human intestine is 70-100% [14].

Caco-2 cells are permeability parameters used to determine drug transport through specialized epithelium derived from human colonic adenocarcinoma which has multiple transport pathways. The parameter categories of Caco-2 cells were >70 nm/sec (high compound permeability); 4-70 nm/sec (medium permeability); <4 nm/sec (low compound permeability) [14], [15]. In Table 2. it can be seen the absorption of the compound active *methyl ricinoleate* has a value of 1.57 log cm/s which is low permeability. Volume of distribution (VDss) is the ability to interact between the distribution phase and the second phase of pharmacokinetics [13]. A compound can be evenly distributed with the same concentration in blood plasma if the volume of distribution is > 0.45 log L/kg, so it can be seen if the compound *methyl ricinoleate* < 0.45 log L/kg, meaning that it is not evenly distributed in blood plasma [15]. Total clearance is the total elimination of a drug concentration, which is at this clearance the concentration of a drug becomesexhausted or does not exist in the body [13]. It can be seen in Table 2. The amount of absorption of the active compound Bouea macrophylla G. tested and released was equally large so that the ADME process that took place was balanced.

The 10 active compounds tested Bouea macrophylla G. (Table 2.) did not act as CYP2D6 inhibitors. Cytochrome P450 is an enzyme that is responsible for the metabolism of a drug. Cytochrome P450 has two main isoforms, namely CYPD26 and CYP3A4 [15]. So if a CYP2D6 inhibitor is found in a compound, it is feared that it will change a drug's pharmacokinetics.

AMES test is a simple way to test the properties of compounds in the form of mutagenic and carcinogenic properties as mutagenic frames [13], [16]. In this study (Table 2.) it was found that all tested Bouea

macrophylla G. compounds were neither mutagenic nor carcinogenic, as was atorvastatin. The safety test of all tested Bouea macrophylla G. active compounds was made by determining the acute and chronic lethal doses as Oral Rate Acute Toxicity (LD50) and Oral Rate Chronic Toxicity (LOAEL) (Table 2), respectively. The LD50 of *methyl ricinoleate* compound is 1,573 mol/kg where the LD50 dose of *methyl ricinoleate* compound is smaller than the LD50 of atorvastatin with a value of 2,877 mol/kg. Meanwhile,the LOAEL value of *methyl ricinoleate* is 2,701 log/mg/kg_bw/day, which means it is lower than the LOAEL for atorvastatin with a value of 4.839 log/mg/kg_bw/day [13].

Table 4. shows that the results of docking angiopoietin-2 (Ang-2) with 10 tested active compounds Bouea macrophylla G. and with atorvastatin resulted in binding affinity varying from -3.3 kcal/mol to -6.5kcal/mol. The binding affinity value has the potential to predict the strength of the interaction between the ligand and the receptor, the more negative or the smaller the affinity value, the more stable and strong the bond is. So it can be seen that the 10 active compounds tested Bouea macrophylla G. have less stable bonds than *atorvastatin* [13], [17].

Hydrogen bonding provides a stronger affinity and plays an important role in stabilizing the structure, so it is very important in the development of drug design. The closer the distance formed in a bond, the stronger the bond and the more significant the activity [13], [18], [19]. Existence of the same residue is very important for the resulting pharmacological effect because the same residue with the reference ligand (comparison drug) is predicted to show activity at the receptor. An active compound is predicted to have a strong bond with the target protein if it is able to bind strongly with hydrogen bonds with the same amino acid residue compared to the control compound (atorvastatin) [20], [21].

Table 4. shows that Tie2 can interact with the Ang-2_ bond of the active leaf compound Bouea macrophylla G. and the Ang-2_*atorvastatin* bond. The presence of protein-to-protein docking interactions is indicated by the amino acid bonds between the ligand and the receptor in the form of hydrogen, hydrophobic, and electrostatic bonds. The type of bond determines the strength of the binding between the drug and the receptor. Bioactive compounds are predicted to have a strong bond with the target receptor if they are ableto bind tightly through hydrogen bonds and bind to one amino acid residue from the active site compared to comparison or inhibitor compounds [22]. The result of docking between Tie2_Ang-2_Vitamin E bonds (Table 4.) resulted in the type of interaction in the form of electrostatic hydrogen. Electrostatic interactions serve to increase the stability of the ligand to the receptor [23].

The binding affinity value of Tie2_Ang-2_*methyl ricinoleate* bond was more negative than that of Ang-2_*methyl ricinoleate* and Tie2_Ang-2_*atorvastatin* (Table 4.). Although the Ang-2_*methyl riconoleate* bond has a weaker binding affinity than the Ang-2_*atorvastatin* bond, the Ang-2_*methyl ricinoleate* bond has the same residue as Ang-2_*atorvastatin* and has one hydrogen bond. Likewise, Tie2_Ang-2_*methyl ricinoleate* which has the same residues with Tie2_Ang-2_*atorvastatin* bonds between the results of protein to protein docking with Ang-2 bonds and 10 active compounds Bouea macrophylla G. and has the most hydrogen bonds between protein to protein docking with bonds Ang-2 and 10 active compounds Bouea macrophylla G. and *atorvastatin*. So that it can be seen if the active compound *methyl ricinoleate* has the most stable and best bond compared to the Ang-2_9 bond of the other active compound Bouea macrophylla
G. tested. This is in line with the research of [13] that a good compound is a compound with a negative binding affinity, has a hydrogen bond, and has the same residue with the protein-ligand bond of comparison. In addition, it is necessary to know if hydrophobic bonds which are non-covalent type bond interactions also play an important role in stabilizing proteins, and increasing the inhibitor's affinity for enzymes [24].

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The existence of hydrogen bonds is very important in a drug development. The hydrogen bonds shared by Ang-2_*methyl ricinoleate* and Tie2_Ang-2_*methyl ricinoleate* play a key role in molecular recognition, structural stability, enzyme catalysis, drug partitioning and permeability [25]. Thus, *methyl ricinoleate* compounds are able to inactivate Ang-2 to block Ang-2/Tie2 signaling as a trigger for inflammation in pathological conditions. The increased expression of Ang-2 under pathological conditions will promote inflammation [26], [27]. During inflammation, Ang-2 is rapidly secreted from the Weibel-Palade body on EC (endothelial cells) and counteracts the vascular stabilizing activity of the Tie2 receptor by blocking its interaction with its canonical agonist ligand, Ang-1. Ang-2 released by exocytosis of WPB (Weibel Palade Body) interferes with the recruitment of pericytes to new blood vessels during angiogenesis by blocking Ang-1/Tie2 autocrine signaling [28- 30]. By inhibiting the Ang-2/Tie2 signaling pathway, vessel-in-vessel growth can be clearly promoted in inflammation. Pericyte coverage which increases after Ang-2 is inhibitedwill affect the stabilization of new blood vessels. VE-Cad accumulation will decrease and phosphorylation will increase. Therefore, by inhibiting Ang-2, the maturation of blood vessels will increase [31]. In addition, the development of a drug needs to pay attention to good pharmacokinetics, the right balance between hydrophilicity and lipophilicity [25]. One indicator that can be used is Lipinski's Rule of Five. *Methyl ricinoleate* can inhibit pro-inflammatory agents such as carrageenan. Thus, the *methyl ricinoleate* compound is able to inactivate Ang-2 to form the process of blocking the Ang-2/Tie2 signal as a trigger for inflammation in pathological conditions. The effect is as an inhibitor of vasodilation, protein extravasation and edema. Thus, based on the results of the study (Table 1.), the *methyl ricinoleate* compound has been declared to have passed and fulfilled the pharmacokinetic requirements.

5. Conclusion

Based on the results of research that has been carried out, the active compound methyl ricinoleate from Bouea macrophylla G. can potentially be a candidate for anti-inflammatory drugs through inhibition of Ang-2.

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