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Submission date: 04-Nov-2021 11:17AM (UTC+0700)
Submission ID: 1692657816
File name: JMFS_ITB.pdf (641.15K)
Word count: 4326
Character count: 25213

Docking Studies and Molecular Dynamics Simulation of Compounds Contained in *Kaempferia Galanga* L. to Lipoxygenase (LOX) for Anti-Inflammatory Drugs

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Abstract. Inflammation is a self-protective response to start the healing process. An anti-inflammatory target worth developing are lipoxygenase inhibitors, which have been studied for several diseases, including severe respiratory disease. This research had the goals of estimating the activity of 21 compounds from K. galanga to inhibit the lipoxygenase (LOX) and estimating the bond stability of the ligand-LOX complex. Based on the compound's affinity for LOX, the compounds in K. galanga were selected by utilizing the PLANTS docking software, with zileuton as the reference ligand. The GROMACS application was used to simulate the molecular dynamics of the LOX-ligand complex at 310 K. Based on the chemPLP score, most of the 21 *K. galanga* compounds showed a higher affinity towards 5-LOX compared to zileuton. *δ*-3-carene had the best affinity for 5-LOX. In the simulation of molecular dynamics until 20 ns, the RMSD of *δ*-3-carene and 5-LOX was not more than 0.03 nm or 0.3 Å, indicating that the whole system showed decent stability and had -1.67392 x 106 kcal/mol as the average potential energy. The results showed that *K. galanga* contains active components of 5-LOX inhibitors that could be developed.

Keywords: *anti-inflammatory; docking; Kaempferia galanga L.; lipoxygenase; molecular dynamics*.

1 Introduction

Kaempferia galanga rhizome is used as a raw material in the traditional medicine industry, especially in Indonesia. It has several functions, for example in the treatment of inflammation as an analgesic, antioxidant, sedative, antimicrobial and even as antineoplastic agent [1]. Essential oil 2.5-4% in the rhizome of K. galanga consists of ethyl cinnamate (25%), ethyl *p*-methoxycinnamate (30%), *p*-methoxycinnamic acid, monoterpenes ketone, and 3-carene-5-one. The other compounds are camphene, *δ*-3-carene, *p*-methoxy styrene, γ-pinene, β-myrcene, *p*-cymene, 1,8-cineole, *iso*-myrcene, camphor, α-terpineol, *p*-cymene- 8-ol, eucarvone and *δ*-cadinene. Some compounds, such as kaempferol, quercetin, cyanidin and delphinidin, can be found in the leaves of

Received August 7th, 2020, Revised February 28th, 2021, Accepted for publication August 15th, 2021.
 ISSN: 2337-5760, DOI: 10.5614/j.math.fund.sci.2021.53.2.4

K. galanga [2]. *K. galanga* has shown potential in inhibiting cyclooxygenase and TNF-alpha [3], and possibly could also bind to lipoxygenase enzyme because of the active site pockets in cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) located near cofactors (Fe^{2+} in 5-LOX or Hem in COX-2). Nevertheless, the single hydrogen bonds in the interaction of α-amyrins with COX-2 and 5-LOX are formed at the different amino acid residues GLN290 of COX-2 and ASN554 of LOX-5 besides hydrophobic interactions [4]. Anti-inflammatory research has been carried out *in vitro* against isolates of *K. galanga* such as isopimarane diterpenoids [3].

Lipoxygenases (LOX) is an oxido-reductase enzyme. Single-electron oxidation by iron atom switching between the redox states of Fe^{2+} and Fe^{3+} occurs in the LOX catalytic reaction. Arachidonic acid is first oxygenated on the fifth carbon by 5-LOX in conjunction with its activating protein, 5-lipoxygenase-activating protein (FLAP), to give 5-hydroperoxy eicosatetraenoic acid (5-HPETE). 5-LOX, with FLAP, and then catalyzes a second step, the dehydration of 5-HPETE to leukotriene [5].

There are two types of drugs for the treatment of inflammation, nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids [6]. Systemic corticosteroids have an integral role in managing inflammatory and immunological conditions. However, these drugs cause serious side effects especially when used at high doses for a long time against conditions such as osteoporosis, adrenal suppression, hyperglycemia, dyslipidemia, cardiovascular disease, Cushing's syndrome, psychiatric disorders, and immunosuppression [7]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to reduce pain and inflammation. Based on the level of selectivity for COX inhibition there are two types of NSAIDs, non-selective and COX-2-selective inhibitors (COXIBS) [8]. Long-term use of NSAIDs causes side effects on the gastrointestinal, renal and cardiovascular systems [9].

Zileuton is a drug that can inhibit 5-LOX, an initial enzyme in the leukotriene pathway. Zileuton is marketed for asthma treatment. This drug has limited clinical use because of its hepatotoxicity [10]. Therefore, new drugs are needed that have greater anti-inflammatory activity and fewer side effects than existing anti-inflammatory drugs. Zileuton could be used as a reference in 5-LOX inhibitors studies.

Virtual screening and drug design optimization have been conducted by molecular docking, a method in computer-aided drug design (CADD) [11]. The physical properties of the structure and how the biological macromolecules function can be discovered through molecular dynamics simulation [12], producing root mean square deviation (RMSD) graphs, root mean square fluctuations (RMSF), and potential energy [13][14]. Several docking studies have been conducted to obtain novel 5-LOX inhibitors, for example docking studies of thiazolyl derivatives [15], diospyrin [16] and coumaperine derivatives [17]. This study conducted molecular dynamic simulations of *K. galanga* compounds as LOX-inhibitor.

2 Materials and Methods

The docking studies in the present study were conducted using the software PLANTS (Protein-Ligand ANT System [18]. The compounds in K. galanga and zileuton in a 3D structure were docked to 5-LOX as receptor. The 3D structure of 5-LOX was downloaded from the Protein Data Bank (PDB) with a resolution of 2.07 Å, PDB code 3V98 [19]. The 5-LOX enzyme was separated from Fe^{2+} ion previously using YASARA (Yet Another Scientific Artificial Reality Application). First, method validation was carried out by redocking Fe^{2+} to the receptor with the requirement that the RMSD value was not more than 2 Å [20]. Marvin Sketch transformed the 3D structure of ligands that are available in PubChem into 2D form. Scanning of the process of protonation was carried out by adjusting the pH to 7.4. The YASARA application was used to find the ligand conformation with the lowest energy before proceeding to molecular docking [18]. The Chemical Piecewise Linear Potential (ChemPLP) scores were obtained from the docking result. A more negative value of the ChemPLP score indicates higher affinity of the compound's bond with the receptor. Visualization of the molecular docking results was performed using Discovery Studio. The compound selected to proceed to the molecular dynamic simulation stage was the compound with the most negative ChemPLP score.

Molecular dynamic simulations were carried out using GROMACS (Groningen Machine for Chemical Simulations) on the compound-receptor complex that was selected in the previous stage. The coordinates of this complex were $x = 9.08400$ Å, $y = 12.12800$ Å and $z = 11.20300$ Å. In the molecular dynamic simulation, the LINCS (Linear Constraint Solver) algorithm was used in the AMBER99SB-ILDN ((Assisted Model Building with Energy Refinement) 99SB side-chain dihedral) force field. Molecular dynamics simulation was conducted for 20 ns with temperature at 310 K. The analytical parameters of the simulation were RMSD (root mean square deviation), RMSF (root mean square fluctuations), and potential energy. The molecular dynamics simulation result was visualized using VMD [21].

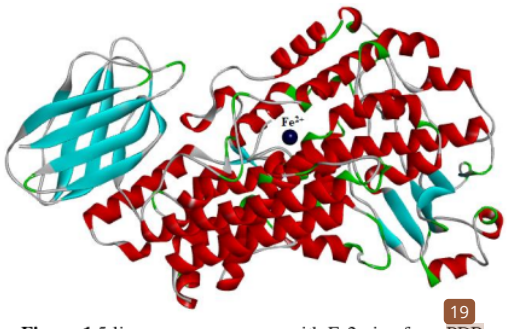

Figure 1 5-lipoxygenase enzyme with Fe2+ ion from PDB.

3 Results and Discussion

3.1 Data Collection

In this study, 21 compounds of *K. galanga* flavonoids that have anti-inflammatory potential were studied for their activity against the lipoxygenase enzyme. The receptor was the 5-lipoxygenase enzyme with Fe^{2+} ion (Figure 1) [19].

The drug used as reference ligand was zileuton, which is a 5-lipoxygenase (5-LOX) inhibitor. It is used in asthma treatment and can be used as a selective tool for evaluation of 5-LOX and leukotriene function [22][23].

Zileuton has been studied for various diseases, such as myocardial infarction, non-alcoholic fatty liver disease, and severe respiratory disease [24]-[28]. In PubChem, the 3D structure of the compounds in *K. galanga* and zileuton were obtained (Table 1) [24].

Table 1 PubChem CID Data of Zileuton and Compounds Contained in K. galanga L.

No	Compound	PubChem CID	No	Compound	PubChem CID
1	Zileuton	60490	12	1,8-cineole	2758
2	Ethyl cinnamate	637758	13	*Iso*-myrcene	519324
3	Ethyl *p*-methoxycinnamic	5281783	14	Camphor	2537
4	*p*-methoxycinnamic acid	699414	15	α-terpineol	17100
5	3-carene-5-one	10057675	16	*p*-cymene-8-ol	7463
6	Camphene	6616	17	Eucarvone	136330
7	*δ*-3-carene	442461	18	*δ*-cadinene	12306054
8	*p*-methoxy styrene	12507	19	Kaempferol	5280863
9	γ-pinene	6431006	20	Quercetin	5280343
10	β-myrcene	31253	21	Cyanidin	128861
11	*p*-cymene	7463	22	Delphinidin	128853

3.2 Docking Study

Docking studies are part of computational chemistry and can be carried out to understand the interactions that occur between ligands and their receptors, which can predict the bonding association [29]. Docking studies can recognize molecules by finding the active sites on the ligand and the receptor that bind to each other, predicting the binding affinity [30]. Molecular docking in this study was carried out using PLANTS for compounds contained in K. galanga and zileuton as reference ligand. PLANTS applies the flexible docking method, i.e. the receptor and the ligand are in a flexible state in the docking process [29]-[31].

Before beginning the docking study, method validation and redocking were carried out using PLANTS with Fe^{2+} ion in order to find out the reliability of the docking protocol. The coordinates of the receptor binding sites were obtained by redocking. These coordinates were used as the default receptor binding sites in the virtual screening for 21 compounds of K. galanga and zileuton [32]. The result of redocking 5-LOX with Fe^{2+} ion produced an RMSD value of 0 Å. The docking method with PLANTS software for 5-LOX as receptor was applicable

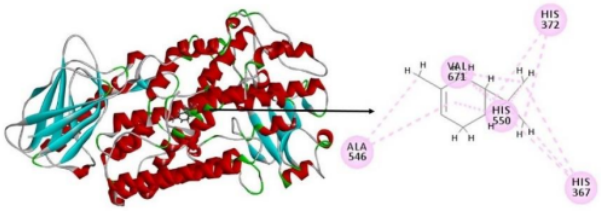

Figure 2 Docking result visualization of δ-3-carene with 5-lipoxygenase complex. δ-3-carene forms 12 hydrophobic bonds with 5-lipoxygenase on the amino acid residues valine 671, alanine 546, histidine 367, histidine 372 and histidine 550.

in this research because the RMSD value of the molecular docking method validation <2 Å [13].

The results are displayed as chemPLP score, which is an empirical fitness function that is optimized for predicting purposes. Among all 21 compounds, the highest affinity was shown by *δ*-3-carene with the lowest chemPLP score, 20.0002 kcal/mol (Figure 2, Table 2), i.e. higher affinity compared to zileuton. There are 12 hydrophobic bonds between *δ*-3-carene and 5-lipoxygenase. *δ*-3-carene and zileuton bind to the same amino acid residue valine 671 in 5-LOX. The interaction types are hydrophobic.

Table 2 Results of Docking Compounds from K. galanga L. and Zileuton to 5-lipoxygenase Enzyme

No	Compound	ChemPLP Score (kcal/mol)	Bond Type	Binding Site
1	Zileuton	-18.2282	Electrostatic	:UNK*:N2 - :ILE673:O
			Hydrogen Bond	:UNK:H2 - :SER670:O
			Hydrophobic	:UNK:C6 - :VAL553
			Hydrophobic	:HIS367 - :UNK:C6
			Hydrophobic	:UNK - :VAL374
			Hydrophobic	:UNK - :VAL671
2	Ethyl cinnamate	-19.9983	Hydrogen Bond	:UNK:H5 - :PHE151:O
			Hydrophobic	:UNK:C5 - :ARG518
3	Ethyl *p*-methoxycinnamic	-19.9923	Hydrogen Bond	:ARG411:HH12 - :UNK:O3
			Hydrogen Bond	:UNK:H7 - :THR403:O
			Hydrogen Bond	:UNK:H8 - :HIS372:O
			Hydrophobic	:UNK:C12 - :ILE415
			Hydrophobic	:TRP147 - :UNK:C12
			Hydrophobic	:PHE151 - :UNK:C12
			Hydrophobic	:HIS372 - :UNK:C9
			Hydrophobic	:UNK - :LEU368
			Hydrophobic	:UNK - :LEU373
			Hydrophobic	:UNK - :ILE415
4	*p*-methoxycinnamic acid	-19.9995	Hydrogen Bond	:ARG370:H - :UNK:O1
			Hydrogen Bond	:UNK:H7 - :ILE365:O
			Hydrophobic	:UNK:C9 - :ARG370
			Hydrophobic	:UNK:C9 - :VAL553
			Hydrophobic	:UNK - :LEU443
			Hydrophobic	:UNK - :LEU448
5	3-carene-5-one	-20.0001	Hydrophobic	:UNK:C5 - :LEU369
			Hydrophobic	:UNK:C5 - :ARG370
			Hydrophobic	:UNK:C5 - :VAL374

No	Compound	ChemPLP Score (kcal/mol)	Bond Type	Binding Site
6	Camphene	-20.0000	Hydrophobic	:UNK:C6 - :VAL374
			Hydrophobic	:UNK:C6 - :LEU448
			Hydrophobic	:UNK:C6 - :MET517
			Hydrophobic	:PHE525 - :UNK:C6
			Hydrophobic	:PRO152 - :UNK
			Hydrophobic	:MET517 - :UNK
			Hydrophobic	:ARG518 - :UNK
			Hydrophobic	:ARG518 - :UNK
			Hydrophobic	:UNK - :PRO152
			Hydrophobic	:UNK - :MET440
			Hydrophobic	:UNK - :MET517
			Hydrophobic	:UNK:C8 - :MET440
			Hydrophobic	:UNK:C8 - :MET517
			Hydrophobic	:UNK:C9 - :PRO152
			Hydrophobic	:UNK:C9 - :MET440
			Hydrophobic	:UNK:C10 - :PRO152
			Hydrophobic	:UNK:C10 - :LEU443
			Hydrophobic	:UNK:C10 - :MET517
7	δ-3-carene	-20.0002	Hydrophobic	:UNK - :VAL671
			Hydrophobic	:UNK:C6 - :VAL671
			Hydrophobic	:UNK:C7 - :VAL671
			Hydrophobic	:UNK:C10 - :ALA546
			Hydrophobic	:ALA546 - :UNK
			Hydrophobic	:HIS367 - :UNK
			Hydrophobic	:HIS367 - :UNK:C6
			Hydrophobic	:HIS367 - :UNK:C7
			Hydrophobic	:HIS372 - :UNK
			Hydrophobic	:HIS372 - :UNK:C7
			Hydrophobic	:HIS550 - :UNK
			Hydrophobic	:HIS550 - :UNK:C6
			Hydrogen Bond	:HIS367:HD1 - :UNK:O1
8	*p*-methoxy styrene	-19.9990	Hydrophobic	:UNK:C8 - :LEU414
			Hydrophobic	:UNK:C8 - :LEU607
			Hydrophobic	:HIS367 - :UNK:C8
			Hydrophobic	:HIS432 - :UNK:C9
			Hydrophobic	:UNK - :LEU368
			Hydrophobic	:UNK - :LEU414
			Hydrophobic	:PRO152 - :UNK
			Hydrophobic	:ILE365 - :UNK
			Hydrophobic	:VAL436 - :UNK
			Hydrophobic	:VAL436 - :UNK
			Hydrophobic	:ALA439 - :UNK
			Hydrophobic	:ALA439 - :UNK
			Hydrophobic	:ALA439 - :UNK:C10
9	γ-pinene	-20.0000	Hydrophobic	:MET440 - :UNK
			Hydrophobic	:UNK - :MET440
			Hydrophobic	:UNK - :LEU443
			Hydrophobic	:UNK:C7 - :ILE365
			Hydrophobic	:UNK:C7 - :VAL436
			Hydrophobic	:UNK:C8 - :LEU373
			Hydrophobic	:UNK:C8 - :VAL436
			Hydrophobic	:UNK:C10 - :LEU369
			Hydrophobic	:UNK:C10 - :MET440
			Hydrophobic	:UNK:C10 - :LEU443
10	β-myrcene	-18.7074	Hydrophobic	:UNK - :LEU373
			Hydrophobic	:UNK:C6 - :MET145
			Hydrophobic	:UNK:C9 - :LEU373
			Hydrophobic	:UNK:C10 - :LEU373

No	Compound	ChemPLP Score (kcal/mol)	Bond Type	Binding Site
11	*p*-cymene	-19.0029	Hydrophobic	:TRP147 - :UNK:C6
			Hydrophobic	:TRP147 - :UNK:C6
			Hydrophobic	:UNK:C1 - :LEU443
			Hydrophobic	:UNK:C10 - :VAL374
			Hydrophobic	:UNK:C10 - :VAL377
			Hydrophobic	:UNK - :VAL377
			Hydrogen Bond	:ARG520:HE - :UNK:O1
			Hydrogen Bond	:ARG520:HH21 - :UNK:O1
12	1,8-cineole	-20.0000	Hydrophobic	:LEU369 - :UNK
			Hydrophobic	:LEU443 - :UNK
			Hydrophobic	:MET517 - :UNK
			Hydrophobic	:UNK - :PRO152
			Hydrophobic	:UNK - :LEU369
			Hydrophobic	:UNK - :LEU443
			Hydrophobic	:UNK:C9 - :LEU443
			Hydrophobic	:UNK:C10 - :MET440
			Hydrophobic	:UNK:C10 - :LEU443
			Hydrophobic	:UNK:C10 - :MET517
			Hydrophobic	:UNK:C9 - :LEU369
			Hydrophobic	:UNK:C9 - :ARG370
13	*Iso*-myrcene	-18.6944	Hydrophobic	:UNK:C10 - :VAL553
			Hydrophobic	:HIS367 - :UNK
			Hydrophobic	:HIS367 - :UNK:C6
			Hydrophobic	:HIS367 - :UNK:C10
			Hydrophobic	:HIS372 - :UNK:C6
			Hydrophobic	:VAL436 - :UNK
14	Camphor	-20.0000	Hydrophobic	:UNK:C8 - :VAL433
			Hydrophobic	:UNK:C8 - :VAL436
			Hydrophobic	:PHE151 - :UNK:C8
			Hydrophobic	:PHE151 - :UNK:C9
			Hydrophobic	:LEU368 - :UNK
			Hydrophobic	:VAL433 - :UNK
			Hydrophobic	:UNK:C8 - :ILE365
			Hydrophobic	:UNK:C8 - :LEU368
15	α-terpineol	-19.9516	Hydrophobic	:UNK:C8 - :LEU369
			Hydrophobic	:UNK:C9 - :MET435
			Hydrophobic	:UNK:C9 - :VAL436
			Hydrophobic	:UNK:C10 - :VAL433
			Hydrophobic	:TRP147 - :UNK:C10
			Hydrophobic	:HIS432 - :UNK
			Hydrophobic	:HIS432 - :UNK:C9
			Hydrophobic	:UNK:C3 - :LEU368
			Hydrophobic	:UNK:C4 - :LEU607
16	*p*-cymene-8-ol	-18.7071	Hydrophobic	:UNK:C10 - :ILE406
			Hydrophobic	:UNK:C10 - :LYS409
			Hydrophobic	:HIS367 - :UNK:C3
			Hydrophobic	:HIS367 - :UNK:C4
			Hydrophobic	:HIS372 - :UNK:C3
			Hydrophobic	:HIS372 - :UNK:C4
			Hydrophobic	:UNK - :ALA410
			Hydrophobic	:PRO152 - :UNK
			Hydrophobic	:VAL374 - :UNK
17	Eucarvone	-20.0001	Hydrophobic	:UNK:C3 - :VAL512
			Hydrophobic	:UNK:C3 - :MET517
			Hydrophobic	:UNK:C4 - :MET517
			Hydrophobic	:UNK:C10 - :VAL374
			Hydrophobic	:UNK:C10 - :LEU448
			Hydrophobic	:UNK:C10 - :MET517

No	Compound	ChemPLP Score (kcal/mol)	Bond Type	Binding Site
18	δ-cadinene	-19.8634	Hydrophobic	:TRP144 - :UNK:C3
			Hydrophobic	:PHE378 - :UNK:C10
			Hydrophobic	:PHE525 - :UNK:C10
			Hydrophobic	:LEU368 - :UNK
			Hydrophobic	:UNK:C5 - :LEU373
			Hydrophobic	:UNK:C5 - :ARG411
			Hydrophobic	:UNK:C14 - :LEU373
			Hydrophobic	:UNK:C15 - :LEU373
			Hydrophobic	:TRP147 - :UNK:C5
			Hydrophobic	:TRP147 - :UNK:C5
			Hydrophobic	:HIS372 - :UNK:C15
19	Kaempferol	-17.7609	Hydrogen Bond	:ARG411:HH11 - :UNK:O1
			Hydrogen Bond	:ARG411:HH12 - :UNK:O1
			Hydrogen Bond	:UNK:O2 - :LEU373:O
			Electrostatic	:ARG411:NH2 - :UNK
			Electrostatic	:GLU376:OE2 - :UNK
			Hydrogen Bond	:ILE155:HN - :UNK:O6
20	Quercetin	-16.9874	Hydrophobic	:TRP147 - :UNK
			Hydrophobic	:TRP147 - :UNK
			Hydrophobic	:UNK - :LEU373
			Hydrogen Bond	:UNK:H7 - :HIS432:O
			Hydrogen Bond	:VAL436:HA - :UNK:O3
21	Cyanidin	-13.3429	Hydrogen Bond	:LEU369:HN - :UNK
			Hydrophobic	:UNK - :ILE365
			Hydrophobic	:UNK - :LEU369
			Hydrophobic	:UNK - :VAL436
			Hydrogen Bond	:UNK:H9 - :THR364:O
22	Delphinidin	-13.3238	Hydrophobic	:LEU373:HD12 - :UNK
			Hydrophobic	:UNK - :LEU369
			Hydrophobic	:UNK - :MET440
			Hydrophobic	:UNK - :MET440

*UNK = Ligand

3.3 Molecular Dynamics Simulation

The stability of the δ-3-carene and 5-lipoxygenase complex was assessed by molecular dynamics simulation (Figure 3). The backbone of both molecules could bend and move easily in the molecular dynamics process [33][34]. The simulation was performed for 20 ns at 310 K.

The relationship between the RMSD value and the time length for the molecular dynamics ascertain the stability of the interactions between the ligand and

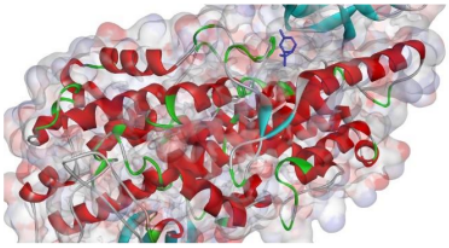

Figure 3 The molecular dynamics visualization for the δ-3-carene with 5-lipoxygenase complex.

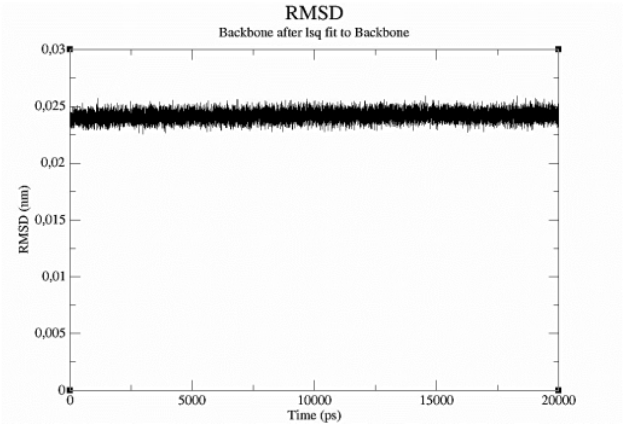

Figure 4 Graph of RMSD changes with time during the molecular dynamic simulation of δ-3-carene with 5-lipoxygenase complex.

receptor molecules. The molecular dynamics result was an RMSD value for the interaction between *δ*-3-carene and 5-lipoxygenase of less than 0.03nm (0.3Å) (Figure 4). This represents a stable complex structure [35]. The RMSF value indicates the atomic movement of the protein during the molecular dynamic simulation. In the RMSF graph, the atoms of the receptor that fluctuate more were the 1st atom (N of serine; RMSF 0.0388 nm), the 263rd atom (C of glycine; RMSF 0.0310nm), the 3045th atom (C of glycine; RMSF 0.0307nm), the 3430th atom (C of glycine; RMSF 0.0316nm), and the 4431st atom (C of glycine; RMSF 0.0309nm) (Figure 5). Potential energy illustrates the energy stability during a molecular dynamic simulation [14]. The graph of the potential energy complex of *δ*-3-carene with lipoxygenase showed energy stability during the simulation with a mean of -1.667392 x 106 kcal/mol (Figure 6).

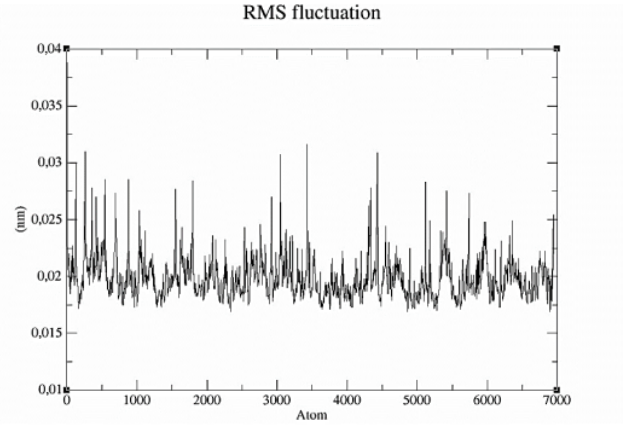

Figure 5 Graph of RMSF in the molecular dynamic simulations of δ-3-carene with 5-lipoxygenase complex.

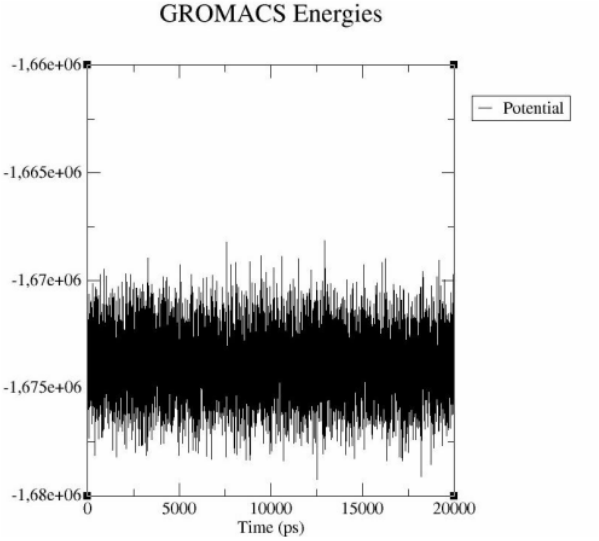

Figure 6 Graph of potential energy in the molecular dynamic simulations of δ-3-carene with 5-lipoxygenase complex.

4 Conclusion

The compound in *K. galanga L.* that has the greatest affinity in inhibiting the enzyme 5-lipoxygenase (5-LOX) is *δ*-3-carene with the most negative ChemPLP value, -20.0002 kcal/mol. The binding affinity of *δ*-3-carene to the 5-LOX receptor is higher than with zileuton as a reference ligand. The complex had good stability during the molecular dynamic simulation with an RMSD and RMSF value smaller than 3Å and -1.67392 x 106 kcal/mol for the mean of potential energy with a simulation time of 20ns.

Acknowledgements

The authors acknowledge the support received from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

References

[1] Umar, M., Asmawi, M., Sadikun, A., Altaf, R., & Iqbal, M.A., *Phytochemistry and Medicinal Properties of Kaempferia Galanga L. (Zingiberaceae) Extracts*, African Journal of Pharmacy and Pharmacology, **5**(14), pp. 1638-1647, 2011. doi:10.5897/AJPP11. 388.

[2] Kumar, A., *Phytochemistry, Pharmacological Activities and Uses of Traditional Medicinal Plant Kaempferia Galanga L. – An Overview*, Journal of Ethnopharmacol, **253**, pp. 112667-112685, 2020. doi:10.1016/j.jep.2020.112667.

[3] Tungcharoen, P., Wattanapiromsakul, C., Tansakul, P., Nakamura, S., Matsuda, H., & Tewtrakul, S., *Anti-Inflammatory Effect of Isopimarane*

Diterpenoids from Kaempferia Galanga, Phytotherapy Research, **34**(3), pp. 612-623. 2020. doi:10.1002/ptr.6549.

[4] Ranjbar, M.M., Assadolahi, V., Yazdani, M., Nikaein, D., & Rashidieh, B., *Virtual Dual Inhibition of COX-2 / 5-LOX Enzymes based on Binding Properties of Alpha-amyrins, the Anti-Inflammatory Compound as a Promising Anti-Cancer Drug*, EXCLI journal, **15**, pp. 238-245. 2016. doi:10.17179/excli2016-164.

[5] Wisastra, R., & Dekker, F.J., *Inflammation, Cancer and Oxidative Lipoxygenase Activity are Intimately Linked*, Cancers, **6**(3), pp. 1500-1521. 2014. doi:10.3390/cancers6031500.

[6] Del Grossi Moura, M., Cruz Lopes, L., Silva, M.T., Barberato-Filho, S., Motta, R., & Bergamaschi, C.C., *Use of Steroid and Nonsteroidal Anti-Inflammatories in the Treatment of Rheumatoid Arthritis: Systematic Review Protocol*, Medicine, **97**(41), pp. e12658. 2018. doi:10.1097/MD.0000000000012658.

[7] Liu, D., Ahmet, A., Ward, L., Krishnamoorthy, P., Mandelcorn, E.D., Brown, J.P., Cohen, A., & Kim, H., *A Practical Guide to the Monitoring and Management of the Complications of Systemic Corticosteroid Therapy*, All Asth Clin Immun, **9**(30), pp. 1-25. 2013. doi:10.1186/1710-1492-9-30.

[8] Harirforoosh, S., Asghar, W., & Jamali, F., *Adverse Effects of Nonsteroidal Antiinflammatory Drugs: An Update of Gastrointestinal, Cardiovascular and Renal Complications*, Journal of Pharmacy & Pharmaceutical Sciences, **16**, pp. 821-47. 2013. doi:10.18433/J3VW2F.

[9] Wong, R.S.Y., *Disease-modifying Effects of Long-term and Continuous Use of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) in Spondyloarthritis*, Adv Pharmacol Pharm Sci, pp. 1-6. 2019. doi:10.1155/2019/5324170.

[10] Joshi, E.M., Heasley, B.H., Chordia, M.D., & Macdonald, T.L., *In vitro Metabolism of 2-Acetylbenzothiophene: Relevance to Zileuton Hepatotoxicity,* Chem Res Toxicol, **17**(2), pp. 137-43. 2004. doi:10.1021/tx0341409. PMID: 14967000.

[11] Chen, Y.C., *Beware of Docking!*, Trends in Pharmacological Sciences. ***36***(2), pp. 78–95, 2015. doi:10.1016/j.tips.2014.12.001.

[12] Karplus, M. & McCammon, J.A., *Molecular Dynamics Simulations of Biomolecules*. Nature Structural Biology, **9**(9), pp. 646-652, 2002. doi:10.1038/nsb0902-646.

[13] Musoev, A., Numonov, S., You, Z., & Gao, H., *Discovery of Novel DPP-IV Inhibitors as Potential Candidates for the Treatment of Type 2 Diabetes Mellitus Predicted by 3D QSAR Pharmacophore Models, Molecular Docking and De Novo Evolution*, Molecules, **24**, pp. 2870-2882, 2019. doi: 10.3390/molecules24162870.

[14] Ajao, A., Kannan, M., Krishna, R., Yakubu, S., Umoh, V., & Ameh, J., *Homology Modeling, Simulation And Molecular Docking Studies Of Catechol-2, 3-Dioxygenase From Burkholderia Cepacia: Involved In Degradation Of Petroleum Hydrocarbons*, Bioinformation, **8**(18), pp. 848-854, 2012. doi:10.6026/97320630008848.

[15] Tsolaki, E., Eleftheriou, P., Kartsev, V., Geronikaki, A., & Saxena, A.K., A*pplication of Docking Analysis in the Prediction and Biological Evaluation of the Lipoxygenase Inhibitory Action of Thiazolyl Derivatives*

of Mycophenolic Acid, Molecules, **23**(7), pp. 1621-49. 2018. doi:10.3390/molecules23071621.

[16] Uddin, G., Rauf, A., Arfan, M., Waliullah, Rehman, T.J., Khan, A.Z., Ali, G., Rehman, B., & Zia-ul-Haq, M., *Molecular Docking of Diospyrin as a LOX Inhibitory Compound*, Journal of Saudi Chemical Society, **20**(SUPPL. 1), pp. S448-S450. 2016. doi:10.1016/j.jscs.2013.01.009.

[17] Muthuraman, S., Sinha, S., Vasavi, C.S., Waidha, K.M., Basu, B., Munussami, P., Balamurali, M.M., Doble, M., & Kumar, R.S., *Design, Synthesis and Identification of Novel Coumaperine Derivatives for Inhibition of Human 5-LOX: Antioxidant, Pseudoperoxidase and Docking Studies*. Bioorganic & Medicinal Chemistry, **27**(4), pp. 604-619. 2019. doi:10.1016/j.bmc.2018.12.043.

[18] Exner, T. E., Korb, O., & Ten Brink, T., *New and Improved Features of the Docking Software PLANTS*, Chemistry Central Journal, **3**(SUPPL. 1), pp. 16, 2009. doi:10.1186/1752-153X-3-S1-P16.

[19] Gilbert, N.C., Rui, Z., Neau, D.B., Waight, M.T., Bartlett, S.G., Boeglin, W.E., Brash, A.R., & Newcomer, M.E., *Conversion of Human 5-Lipoxygenase to a 15-Lipoxygenase by a Point Mutation to Mimic Phosphorylation at Serine-663*, The FASEB Journal, **26**(8), pp. 3222-3229, 2012. doi:10.1096/fj.12-205286.

[20] Atiatul, M., Marlyn Dian, L., Dwi, H., & Parsaoran, S., *Molecular Docking of Interaction between E-Cadherin Protein and Conformational Structure of Cyclic Peptide ADTC3 (Ac-CADTPC-NH2) Simulated on 20 ns*, Jurnal Kimia Sains dan Aplikasi, **20**(1), pp. 30-36, 2017. doi:10.14710/jksa.20.1.30-36.

[21] Hu, C. & Ma, S., *Recent Development of Lipoxygenase Inhibitors as Anti-Inflammatory Agents*, Medchemcomm, **9**(2), pp. 212-225, 2018. doi:10.1039/c7md00390k.

[22] Liu, Y., Wang, W., Li, Y., Xiao, Y., Cheng, J., & Jia, J., *The 5-Lipoxygenase Inhibitor Zileuton Confers Neuroprotection against Glutamate Oxidative Damage by Inhibiting Ferroptosis*, Biological and Pharmaceutical Bulletin, **38**(8), pp. 1234-1239, 2015. doi:10.1248/bpb.b15-00048.

[23] Rossi, A., Pergola, C., Koeberle, A., Hoffmann, M., Dehm, F., Bramanti, P., Cuzzocrea, S., Werz, O., & Sautebin, L., *The 5-Lipoxygenase Inhibitor, Zileuton, Suppresses Prostaglandin Biosynthesis by Inhibition of Arachidonic Acid Release in Macrophages*, British journal of pharmacology, **161**(3), pp. 555-570, 2010. doi:10.1111/j.1476-5381.2010.00930.x.

[24] Berger, W., De Chandt, M.T., & Cairns, C.B., *Zileuton: Clinical Implications of 5-Lipoxygenase Inhibition in Severe Airway Disease*, International Journal of Clinical Practice, **61**(4), pp. 663-676, 2007. doi:10.1111/j.1742-1241.2007.01320.x.

[25] Abueid, L., Uslu, Ü., Cumbul, A., Velioğlu Öğünç, A., Ercan, F., & Alican, İ., *Inhibition of 5-Lipoxygenase by Zileuton in a Rat Model of Myocardial Infarction*, Anatolian Journal of Cardiology, **17**(4), pp. 269-275, 2017. doi:10.14744/AnatolJCardiol.2016.7248.

[26] Bruno, F., Spaziano, G., Liparulo, A., Roviezzo, F., Nabavi, S.M., Sureda, A., Filosa, R., & D'Agostino, B., *Recent Advances in the Search for Novel 5-Lipoxygenase Inhibitors for the Treatment of Asthma*,

European Journal of Medicinal Chemistry, **153**, pp. 65-72, 2018. doi:10.1016/j.ejmech.2017.10.020.

[27] Laidlaw, T.M., Fuentes, D.J., & Wang, Y., *Efficacy of Zileuton in Patients with Asthma and History of Aspirin Sensitivity: A Retrospective Analysis of Data from Two Phase 3 Studies*, Journal of Allergy and Clinical Immunology, **139**(2), pp. AB384, 2017. doi:10.1016/j.jaci.2016.12.924.

[28] Liu, Y., Wang, W., Li, Y., Xiao, Y., Cheng, J., & Jia, J., T*he 5-Lipoxygenase Inhibitor Zileuton Confers Neuroprotection against Glutamate Oxidative Damage by Inhibiting Ferroptosis*. Biological and Pharmaceutical Bulletin, **38**(8), pp. 1234-1239, 2015. doi:10.1248/bpb.b15-00048.

[29] Morris, G.M. & Lim-Wilby, M., *Molecular docking*, Molecular Modeling of Proteins, Humana Press, pp. 365-382), 2008.

[30] Xuan-Yu, M., Hong-Xing, Z., Mihaly, M., & Meng, C., *Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery*. Current Computer-Aided Drug Design, **7**(2), pp. 146-157, 2011. doi:10.2174/157340911795677602.

[31] Halperin, I., Ma, B., Wolfson, H., & Nussinov, R., *Principles of Docking: an Overview of Search Algorithms and a Guide to Scoring Functions*. Proteins: Structure, Function, and Bioinformatics, **47**(4), pp. 409-443, **2002**. doi:10.1002/prot.10115.

[32] Korb, O., Stützle, T., & Exner, T.E., *An Ant Colony Optimization Approach to Flexible Protein-Ligand Docking*, Swarm Intelligence, **1**(2), pp. 115-134. 2007. doi:10.1007/s11721-007-0006-9.

[33] Pronk, S., Páll, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M.R., Smith, J.C., Kasson, P.M., van der Spoel, D., & Hess, B., *GROMACS 4.5: A High-Throughput and Highly Parallel Open Source Molecular Simulation Toolkit*, Bioinformatics, **29**(7), pp. 845-854. 2013. doi:10.1093/bioinformatics/btt055.

[34] Van Dijk, M., Ter Laak, A.M., Wichard, J.D., Capoferri, L., Vermeulen, N.P.E., & Geerke, D.P., *Comprehensive and Automated Linear Interaction Energy Based Binding-Affinity Prediction for Multifarious Cytochrome P450 Aromatase Inhibitors*, Journal of Chemical Information and Modeling, **57**(9), pp. 2294-2308, 2017. doi:10.1021/acs.jcim.7b00222.

[35] Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., & Berendsen, H.J.C., *GROMACS: Fast, Flexible, and Free*, Journal of Computational Chemistry, **26**(16), pp. 1701-1718, 2005. doi:10.1002/jcc.20291.

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