https://doi.org/10.46344/JBINO.2021.v10i06.12

MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATION CHEMICAL COMPOUNDS IN *CURCUMA LONGA* AS AN ALTERNATIVE ANTI-INFLAMMATION AGAINST CYCLOOXYGENASE ENZYME TARGET

Purwaniati, Amelliyani, Fauzan Zein Muttaqin

Pharmaceutical Analysis and Medicinal Chemistry, Pharmacy Faculty, Universitas Bhakti Kencana, Jl. Soekarno-Hatta 754 Bandung, Indonesia

Email: purwaniati@bku.ac.id

ABSTRACT

Turmeric is an ingredient commonly used in traditional medicine. Various studies have proven that one of its properties is anti-inflammatory. The content of curcuminoids and essential oils is thought to play a role in this activity. This study was conducted to find lead compounds in anti-inflammatory drugs that work by inhibiting the activity of the cyclooxygenase (COX) enzyme. This study involved COX-1 and COX-2. The target protein structure used is the COX structure obtained from Protein Data Bank with ID 1EQG for COX-1, and 4PH9 for COX-2. Molecular docking and molecular dynamics simulation were chosen as the methods in this study. This study proves that there are at least 6 compounds in turmeric that can be lead compounds as an anti-inflammatory. Cyclocurcumin, dihydrocurcumin, and 5'-methoxycurcumin provide stronger affinity than natural ligands for COX-1. Cyclocurcumin is even predicted to have a better affinity than the comparison drug (ketorolac). These three compounds can be anti-inflammatory lead compounds in COX-1. Meanwhile, curcumin, demethoxycurcumin, and bisdemethoxycurcumin were shown to have a good affinity for COX-2. The affinity values are slightly higher than the natural ligands, but not higher than the comparison drug (celecoxib). So these compounds are very promising to be used as COX-2 selective anti-inflammatory lead compounds. These compounds must be further optimized to be used as anti-inflammatory drugs. So that the candidate drug molecules with the appropriate pharmacokinetic and pharmacodynamic profiles are obtained.

Keywords: anti-inflammation, cyclooxygenase enzyme, curcuminoid, turmeric

INTRODUCTION

Inflammation is a protective mechanism of the body that indicates the presence of interference or the entry of foreign objects into the body. This condition can cause tissue damage. Inflammation is always accompanied by an unpleasant response. Inflammation can occur due to trauma, exposure to certain chemicals, and heat. Inflammation can occur by involving various physiological and pathological processes (Medzhitov, 2008).

Based on the duration of the inflammation, it is divided into acute and chronic inflammation. Various studies show that inflammation is crucial to the development of various diseases such as diabetes, cancer, autoimmune, cardiovascular, and others. Acute inflammation lasts from minutes to days. This inflammation is related to innate immunity (Arulselvan et al., 2016). Acute inflammation occurs due to leakage of protein or plasma fluid and leukocyte migration to extravascular areas (Kobayashi et al., 2014).

Chronic inflammation occurs in patients with diseases caused by infections, chronic arterial disease (Toker et al., 2005), myocardial ischemia (Anselmi et al., 2004), Alzheimer's, stroke, cancer, hypertension, and others. A study also shows that depression greatly determines a person's inflammatory status (Arulselvan et al., 2016).

Cyclooxygenase (COX) is an enzyme that plays a very important role in the inflammatory process. COX consists of two isoforms, namely COX-1 and COX-2. Inhibition of activity on COX-2 is the most desirable mechanism of action of a non-steroidal anti-inflammatory drug (NSAID). The ratio of inhibition of COX-1 and COX-2 will determine the side effects of the drug. Several NSAIDs are also thought to act on the lipoxygenase pathway (Cashman, 1996). COX-2 is the main enzyme that plays a role in fatty acid metabolism. This enzyme is induced by proinflammatory cytokines that produce prostaglandins. Under inflammatory conditions, COX-2 is upregulated in inflammatory conditions. Prostaglandins will further stimulate the proliferation of cancer cells, increase the risk of angiogenesis, inhibit apoptosis and increase metastasis (Shreena J. Desai, Ben Prickril, 2018).

Curcumin as one of the ingredients in turmeric has been shown to have anti-inflammatory and analgesic activity. Unfortunately, these molecules have low bioavailability (Lee et al., 2013). Curcumin is not the only compound present in turmeric. There are many other chemical constituents. This study was conducted to examine the potential compounds as anti-inflammatory lead compounds from turmeric. Molecular docking studies and molecular dynamics simulations were selected for this determination.

MATERIALS AND METHODS

Materials

The materials used include 3D COX-1 structures with code 4PH9 and COX-2 with code 1EQG obtained from the Protein Data Bank and accessed on www.rcsb.org in PDB file format. The two target proteins form a complex with ibuprofen as a natural ligand. The test ligand is the 3D structure of the 12 compounds found in turmeric.

Tools

The tools used include hardware and software. Hardware in the form of a notebook and a computer. Notebook

specification is Processor type Intel® Pentium Inside™, 2 GB RAM, and 240 GB SSD. While the computer with Processor Intel® XEON®CPU E5-2620 v4@2.10GHz x 16, VGA: Graphics GeForce GTX 1080/PCIe/SSE2, RAM 64 GB, OS: Linux Ubuntu 16.04 LTS, HDD: 2 TB. The software consists of a Windows™ operating system capable of molecular docking including ChemOffice 2014, Gaussian 09, Gaussian 0.5 view, Autodock version 4.2.6, and Discovery Studio Visualizer 2016. The software used to perform molecular dynamics simulations of the operating system Linux is AMBER.

Preparation of target protein and test ligand

The target protein complex and natural ligand COX-2 with PDB ID 4PH9 (Benjamin J. Orlando, Michael J Lucido, 2015) and COX-1 with PDB ID 1EQG (Selinsky et al., 2001) were downloaded from rcsb.org. The protein is then separated from the water molecule and its natural ligands with Discovery Studio Visualizer application. The structure of the protein and natural ligands that have been separated can be seen in Figure 1.

The 2D and 3D structures of the test ligands were modeled using the ChemDraw Ultra and Chem3D 2014 applications. Geometry optimization was carried out using the Gaussian09 application.

Molecular Docking and Molecular Dynamic Simulation

The molecular docking study begins with the validation of the docking method. A valid docking method is then used to dock the test ligands. The test ligand that gave good binding affinity to both targets was then continued with molecular dynamics simulations. Molecular docking was carried out with the Autodock 4.2.6 application and molecular dynamics simulation with the Amber application.

RESULTS AND DISCUSSION

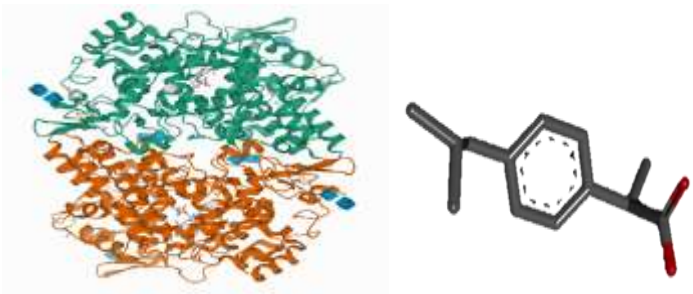
The molecular structure of the COX-1 and COX-2 targets that have been separated can be seen in Figure 1. Both targets have the same natural ligand, namely ibuprofen. In the validation of the docking method, the protein structure and natural ligands that have been separated are then docked again. The docking method is declared valid if it provides an RMSD value less than 2 Å. The docking parameters of the valid docking method for both targets can be seen in Table 1. The docking method used in this study is the Lamarckian Genetic Algorithm with a number of GA runs 100 and a number of evals medium. The results can be seen in Table 3, while the interactions can be seen in Figure 3. Based on Figure 3, it can be seen that ibuprofen (natural ligand) forms 3 hydrogen bonds with COX-1. Whereas in COX-2, the natural ligand interacts with the target protein through 2 hydrogen bonds and an ionic bond. In addition to hydrogen and ionic bonds, ligands also interact weakly through van der Waals interactions and other weak interactions. In COX-1 there are at least 16 amino acids involved in its interaction with ibuprofen. Whereas in COX-2 there are at least 17 amino acids involved in its interaction with natural ligands. These amino acids are amino acids found at each binding site. The amino acid residues in COX-1 include LEU A:384, LEU A:352, SER A:353, TYR A:355, ARG A:120, VAL A:116, LEU:531, LEU A:359, ALA A:527, ILE A:523, VAL A:349. SER A:530, MET

A:522, TRP A:387, PHE A:518, and GLY A:526. Meanwhile, COX-2 includes GLY A:527, SER A:531, PHE A:382, LEU A:385, PHE A:519, MET A:523, LEU A:353, VAL A:524, TRP A: 388, ALA A:528, VAL A:350, SER A:354, TYR A:356, ARG A:121, LEU A:360, VAL A:117, and LEU A:532.

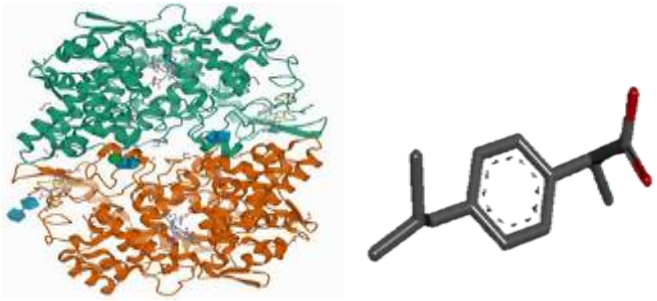
This study involved 12 test ligands, as can be seen in Figure 2. This study also used comparison ligands. Ketorolac was used as a comparison for COX-1, and celecoxib was used as a comparison for COX-2. The two comparators were selected based on their selectivity for each target. Ketorolac is highly selective on COX-1 (Lashbrook et al., 1999) and celecoxib is highly selective on COX-2 (Alsayed et al., 2017).

The docking result of the test ligands can be seen in Table 4. Based on the data in Table 4 it can be seen that the comparison compound used was correct. Ketorolac has a much better affinity for COX-1 than for COX-2. In contrast, celecoxib has a much stronger affinity for COX-2 than for COX-1. Although it has a higher affinity for COX-2, celecoxib also has a relatively high affinity for COX-1. Ibuprofen as a natural ligand on both targets has almost the same affinity for both targets. L4, L5, and L6 were predicted to have better affinity than natural ligands for COX-1, and only L4 was predicted to have a stronger affinity than ketorolac. L1, L2, and L3 has slightly better affinity than natural ligands but is still lower than their COX-2 counterpart. Visualization of the interaction that occurs between L6 on COX-2 and L1 on COX-2 can be seen in Figure 4. The visualization shows that the position of the test ligand is indeed docked at the binding site. this can be proven through the interactions that occur between the ligands and the amino acids that are present at the binding site.

Ligands that showed better affinity than natural ligands were tested for interaction stability through molecular dynamics simulations. The simulation lasted for 50 ns. L4, L5, L6, and ketorolac were simulated on COX-1. Meanwhile, L1, L2, L3, and celecoxib were simulated on COX-2. RMSD and RMSF simulation results can be seen in Figure 5. The RMSD and RMSF graphs for both COX-1 and COX-2 natural ligands (ibuprofen) show the stability of their interactions since the beginning of the simulation. Likewise, the comparison, namely ketorolac on COX-1 and celecoxib on COX-2 showed the stability of their interactions since the beginning of the simulation.



a).



b)

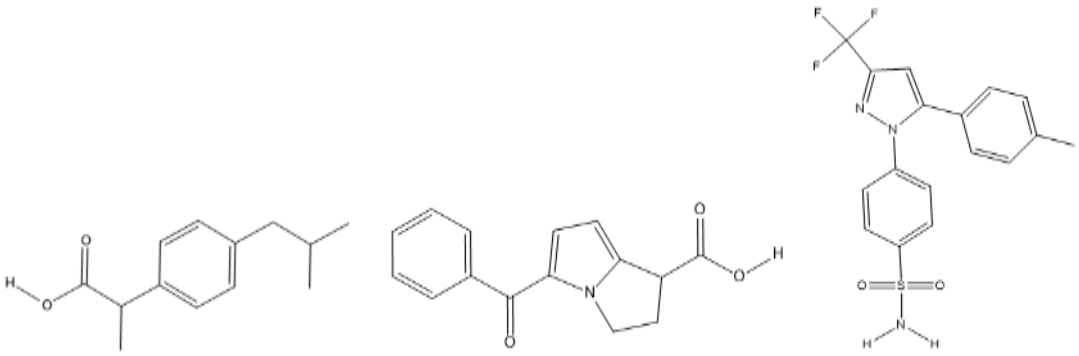
Figure 1. 3D structure of the target protein and its natural ligands, a). COX-1; b)COX-2

Tabel 1. The valid docking parameters on COX-1 and COX-2

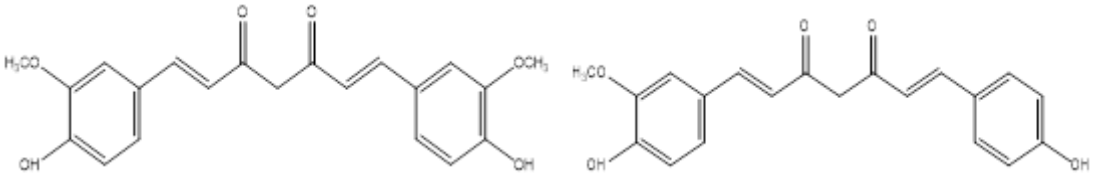
Grid Box			Grid Spacing	Grid Center		
COX-1						
X	Y	Z	0,375Å	X Center	Y Center	Z Center
40	40	40		26.643	33.106	200.231
COX-2						
X	Y	Z	0,375Å	X Center	Y Center	Z Center
60	58	62		13.773	23.244	25.513

Tabel 3. The results of docking method validation

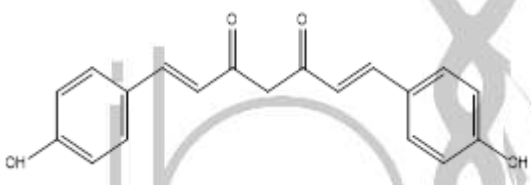
	COX-1	COX-2
ΔG	-8,03 kcal/mol	-8,24 kcal/mol
Ki	1300 nM	917.51 nM
Run	44	39
RMSD	0,98 Å	0,89 Å



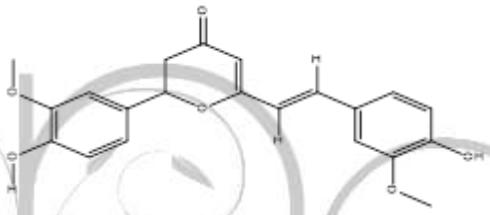
a) b) c)



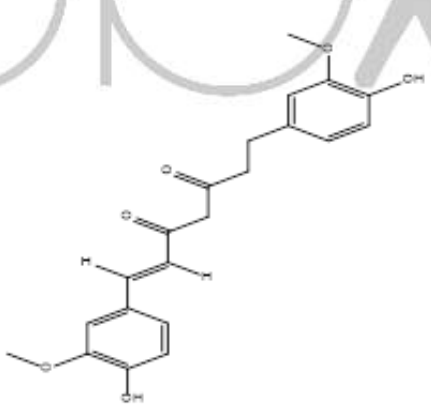
L1 L2



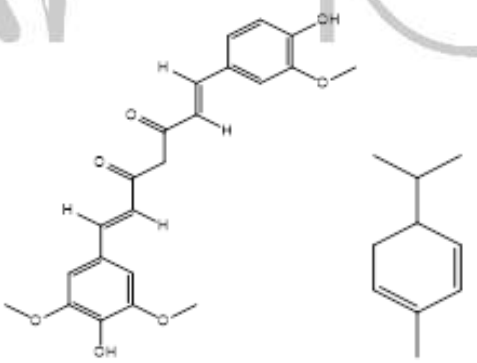
L3



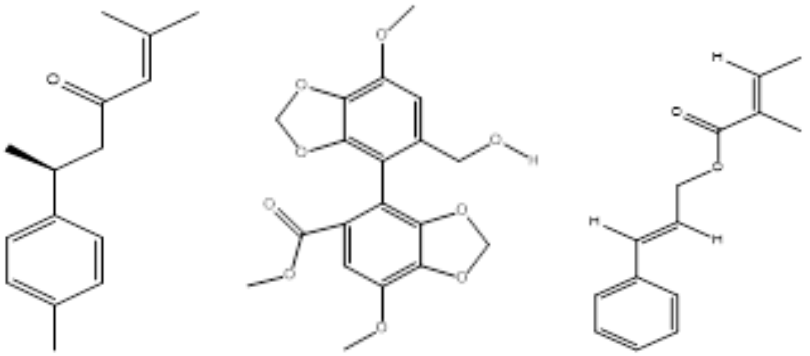
L4



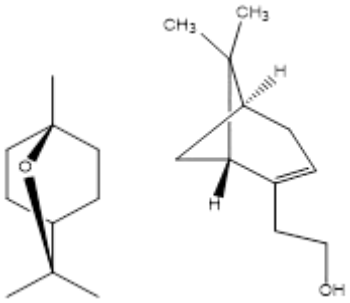
L5



L6 L7

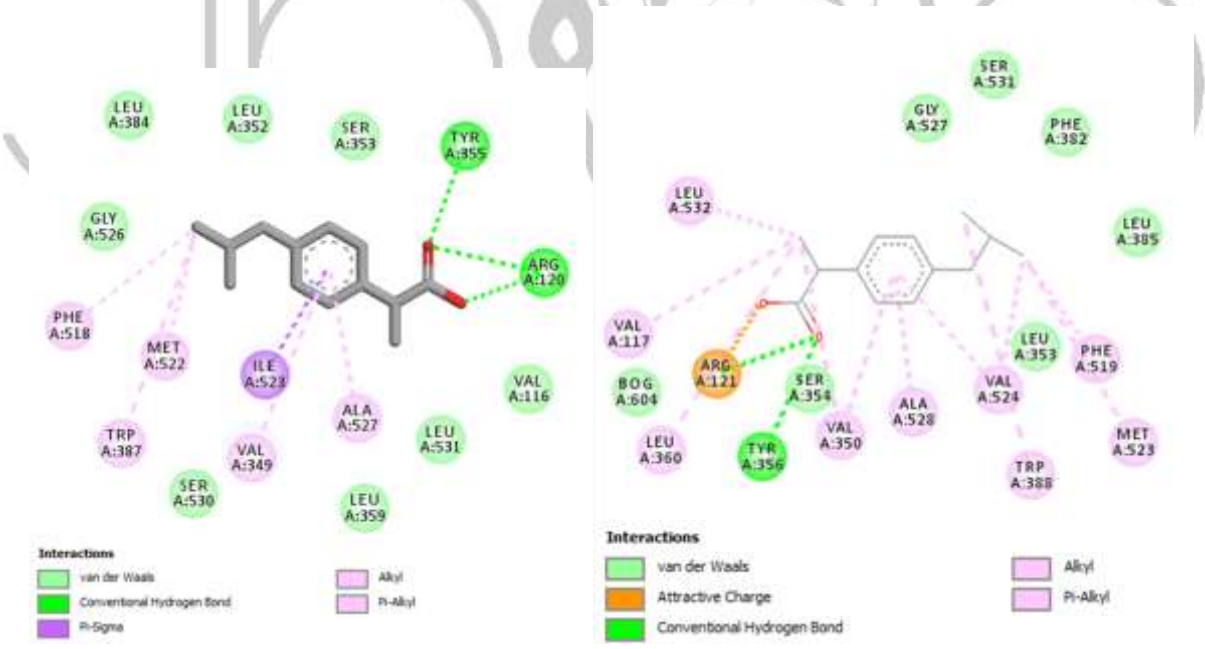


L8 **L9** **L10**



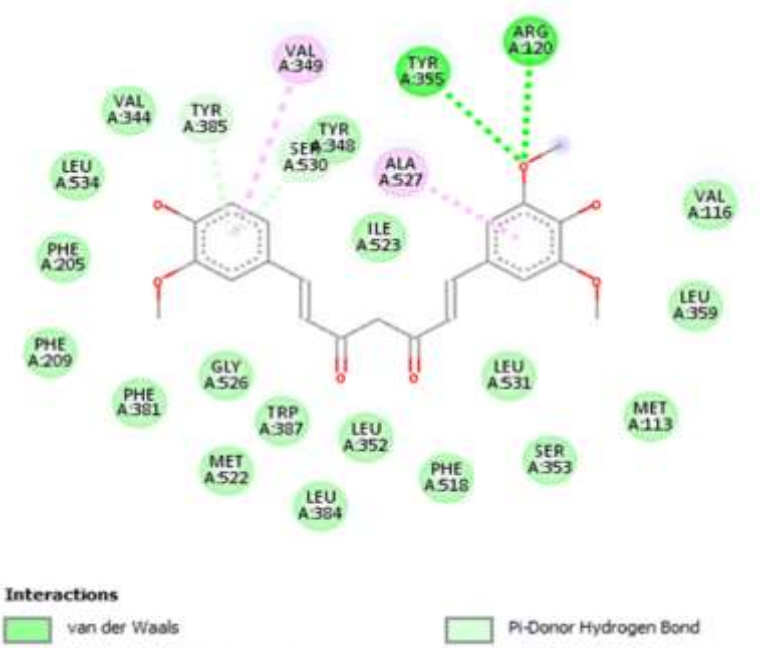
L11 **L12**

Figure 2. Structure of a). natural ligand (ibuprofen); b). Comparison drug of COX-1 (ketorolac); c). Comparison drug of COX-2 (celecoxib). LI-L12 are test ligands, LI: curcumin, L2: demethoxycurcumin, L3: bisdemethocycurcumin, L4: cyclocurcumin, L5: dihyrocurcumin, L6: 5'-methoxycurcumin, L7: 1-phellandrene, L8: ar-turmeron, L9: bicyclol, L10: cinnamyl tiglate, L11: eucalyptol, L12: methylol pinene

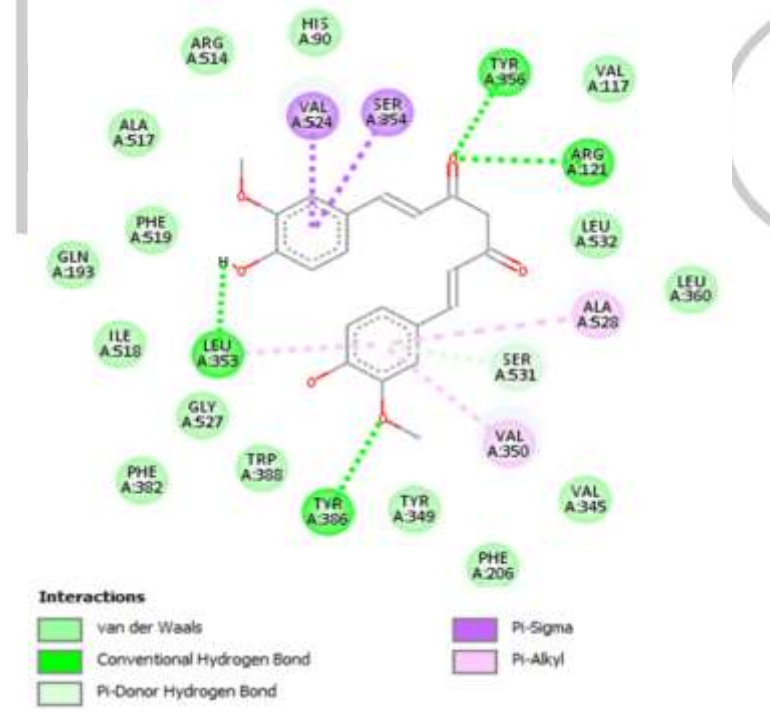


a) b)

Figure 3. Visualization of the interaction between the target protein and its natural ligand (a). COX-1; (b). COX-2



a)



b)

Figure 4. The interaction of the test ligand on the target protein: a). L6 interaction on COX-1, b). L1 interaction on COX-2

Table 4. Docking result of the natural ligand, comparison, and the test ligands

Ligand	COX-1		COX-2	
	Binding energy (Kcal/mol)	Ki (nM)	Binding energy (Kcal.mol)	Ki (nM)
Natural ligand	-8.03	1300	-8.24	917.51
Celecoxib	-9.21	176.06	-11.32	5.04
Ketorolac	-8.64	465.4	-5.21	117330
L1	-8.00	1370	-9.04	236.78
L2	-7.69	2300	-8.45	644.42
L3	-7.82	1860	-8.33	777.14
L4	-8.73	398.7	-7.02	4,930.00
L5	-8.27	864.9	-5.57	82,870.00
L6	-8.06	1230	-7.85	1,750.00
L7	-5.99	40640	-5.98	41,260.00
L8	-7.63	2570	-7.34	4,170.00
L9	-6.73	11610	-5.79	57,410.00
L10	-7.78	1990	-7.33	4,210.00
L11	-6.07	35830	-6.17	30,180.00
L12	-6.29	24340	-5.97	41,860.00

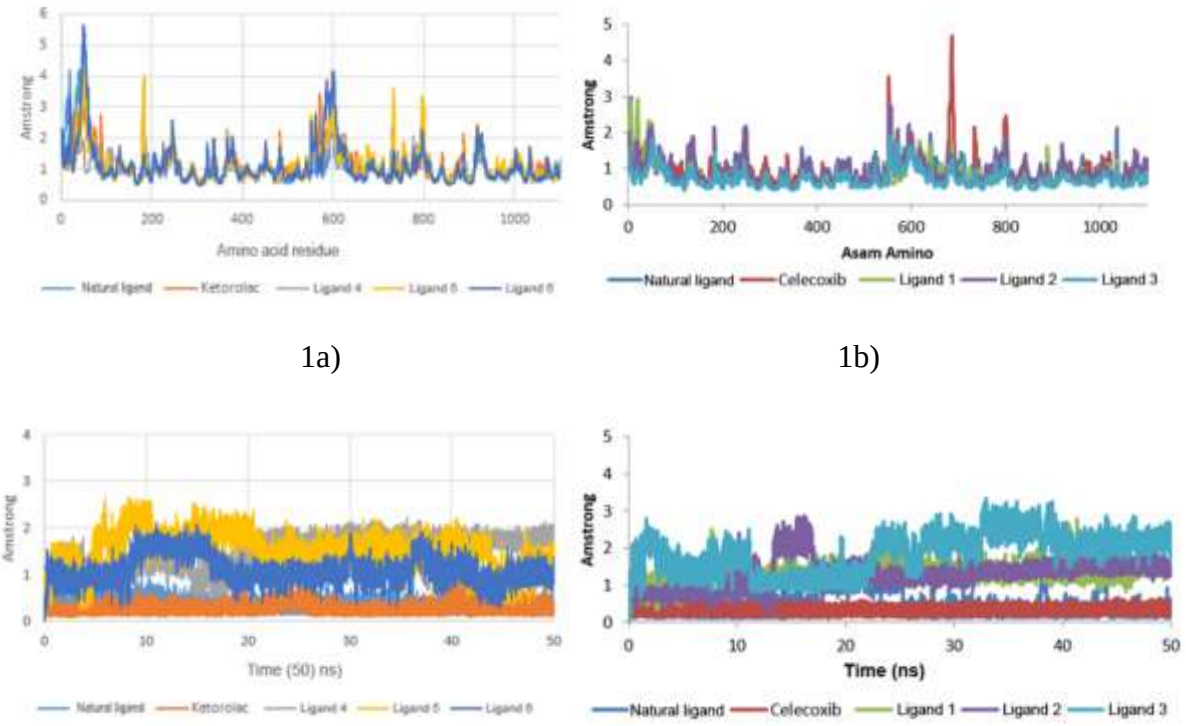


Figure 5. RMSF and RMSD molecular dynamic simulation Result: 1a). RMSF on COX-1, 1b). RMSF on COX-2, 2a). RMSD on COX-1, and 2b). RMSD on COX-2

CONCLUSION

This study proves that several compounds contained in turmeric deserve to be developed the anti-inflammatory. L1 (*curcumin*), L2 (*demethoxycurcumin*), and L3 (*bisdemethocycurcumin*) the potential as COX-2 selective anti-inflammatory. Meanwhile, L4 (*cyclocurcumin*), L5 (*dihyrocurcumin*), and L6 (*5'-methoxycurcumin*) have potential as selective anti-inflammatory agents on COX-1.

ACKNOWLEDGMENT

The author would like to thank *Lembaga Penelitian dan Pengabdian kepada Masyarakat* (LPPM), Universitas Bhakti Kencana, which has funded this research activity.

REFERENCES

Alsayed, S. S. R., Elshemy, H. A. H., Abdelgawad, M. A., Abdel-Latif, M. S., & Abdellatif, K. R. A. (2017). Design, synthesis and biological screening of some novel celecoxib and etoricoxib analogs with promising COX-2 selectivity, anti-inflammatory activity and gastric safety profile. In Bioorganic Chemistry (Vol. 70). https://doi.org/10.1016/j.bioorg.2016.12.008

Anselmi, A., Abbate, A., Girola, F., Nasso, G., Biondi-Zoccai, G. G. L., Possati, G., & Gaudino, M. (2004). Myocardial ischemia, stunning, inflammation, and apoptosis during cardiac surgery: A review of evidence. European Journal of Cardio-Thoracic Surgery, 25(3), 304–311. https://doi.org/10.1016/j.ejcts.2003.12.003

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of Antioxidants and Natural Products in Inflammation. Oxidative Medicine and Cellular Longevity, 2016. https://doi.org/10.1155/2016/5276130

Benjamin J. Orlando, Michael J Lucido, M. G. M. (2015). The structure of ibuprofen bound to cyclooxygenase-2. J Struct Biol., 23(1), 1–7. https://doi.org/10.1016/j.jsb.2014.11.005

Cashman, J. N. (1996). The mechanisms of action of NSAIDs in analgesia. Drugs, 52(SUPPL. 5), 13–23. https://doi.org/10.2165/00003495-199600525-00004

Kobayashi, H., Higashiura, Y., Shigetomi, H., & Kajihara, H. (2014). Pathogenesis of endometriosis: The role of initial infection and subsequent sterile inflammation (Review). Molecular Medicine Reports, 9(1), 9–15. https://doi.org/10.3892/mmr.2013.1755

Lashbrook, J. M., Ossipov, M. H., Hunter, J. C., Raffa, R. B., Tallarida, R. J., & Porreca, F. (1999). Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX1- and COX2-inhibitors in nerve-injured rats. Pain, 82(1), 65–72. https://doi.org/10.1016/S0304-3959(99)00031-7

Lee, J. Y., Shin, T. J., Choi, J. M., Seo, K. S., Kim, H. J., Yoon, T. G., Lee, Y. S., Han, H., Chung, H. J., Oh, Y., Jung, S. J., & Shin, K. J. (2013). Antinociceptive curcuminoid, KMS4034, effects on inflammatory and neuropathic pain likely via modulating TRPV1 in mice. British Journal of Anaesthesia, 111(4), 667–672. https://doi.org/10.1093/bja/aet176

Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature, 454(7203), 428–435. https://doi.org/10.1038/nature07201

Selinsky, B. S., Gupta, K., Sharkey, C. T., & Loll, P. J. (2001). Structural analysis of NSAID binding by prostaglandin H2 synthase: Time-dependent and time-independent inhibitors elicit identical enzyme conformations. Biochemistry, 40(17), 5172–5180. https://doi.org/10.1021/bi010045s

Shreena J. Desai, Ben Prickril, and A. R. (2018). Mechanisms of phytonutrient modulation of Cyclooxygenase-2 (COX-2) and inflammation related to cancer Shreena. Physiology & Behavior, 70(1), 1–40. https://doi.org/10.1080/01635581.2018.1446091.Mechanisms

Toker, S., Shirom, A., Shapira, I., Berliner, S., & Melamed, S. (2005). The association between burnout, depression, anxiety, and inflammation biomarkers: C-reactive protein and fibrinogen in men and women. Journal of Occupational Health Psychology, 10(4), 344–362. https://doi.org/10.1037/1076-8998.10.4.344