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FULL PAPER

In silico study of anthocyanin and ternatin flavonoids for the treatment of inflammation-related diseases using molecular docking analysis

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Abstract

Inflammatory markers such as cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO), and prostaglandin (PEG) are widely known as major targets in discovering natural anti-inflammatory drugs for the treatment of inflammation-related diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and aspirin are mostly used at present, however, some NSAIDS have been reported to cause gastrointestinal side effect due to ligand-protein interaction. Molecular docking is a promising tool to study such modes of interaction. In this study, we evaluated the potential use of anthocyanin and ternatin flavonoids as natural anti-inflammatory agents for treatment of inflammatory-related diseases using *in silico* molecular docking assay. Automated docking study using Protein-Ligand ANT System (PLANTS) and AutoDock Vina was performed with various ligand molecules, including ibuprofen, anthocyanin, and ternatin against the protein crystal structures of COX-1, COX-2, iNOS, and MPO. The *in silico* data demonstrated that ibuprofen bound effectively to the active site of COX-1 and MPO with minimum binding energy, yet the compound required more energy to bind the active site of COX-2. Ternatin flavonoid was bound to COX-2 and iNOS with minimum binding energy. In terms of binding energy, anthocyanin flavonoid was found to be effective for inhibiting COX-1, COX-2, and iNOS. These results suggested that anthocyanin and ternatin flavonoids may potentially be developed as anti-inflammatory drug candidate for the treatment of inflammatory-related diseases.

1. Introduction

The cyclooxygenase (COX) activity is one of the major targets of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, naproxen, and indomethacin. NSAIDs are common use drugs, both economically and clinically, but have the gastric side effect. COX-2 has more attention to the development of selective inhibitors, such as celecoxib and rofecoxib (Selinsky *et al*., 2001; Liu *et al.,* 2019). Several reports on NSAIDs noted that about 20% of regular users of NSAIDs may cause duodenum or gastric ulcer (Wallace, 2001). Flavonoids consist of various classes such as anthocyanins, flavonols, flavones, flavanones, and isoflavones that have been reported to possess anti-inflammatory activities. Butterfly pea (*Clitoria ternatea)* blue petal riched in ternatin anthocyanin has ability to suppress the nuclear factor kappa beta (NF-kB) translocation and inducible nitric oxide synthase (iNOS) expression in macrophages. In addition, *C. ternatea* ternatin anthocyanin can act as a drug or nutraceutical agent for protection against chronic inflammatory diseases by suppressing the excessive production of pro-inflammatory mediators secreted by macrophages, and as a promising candidate for cancer therapeutics (Nair *et al*., 2015; Srinivas *et al.*, 2019). A study by Bukhari *et al*. (2015) demonstrated that flavonoids such as chalcone and pyrazoline exerted anti-inflammatory activity by attenuating the expression of *iNOS, COX-2*, and other pro-inflammatory cytokines.

C. ternatea plant also exerts antioxidant property as a defense system to scavenge the reactive oxygen species (ROS) and protect the plant from destructive reactions (Sivaprabha *et al*., 2008). Antioxidant compounds in *C. ternatea* belong to anthocyanins that are known as phenolic antioxidants. Antioxidant and ROS have diverse roles in plant growth and in resistance to environmental stress (Zhang, 2019). ROS are continuously produced in plants as toxic byproducts of aerobic metabolism and rapidly detoxified. Some antioxidants are themselves free radical, donating

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electrons and neutralize the toxic free radicals.

Molecular docking is a computational chemistry method to study the rational drug design process. For example, this assay could be applied for studying ligand-receptor interaction mode and exploring several chemical data through *in silico* studies. Docking result using the software could be used to predict about binding energy of enantiomers (Ramírez and Caballero, 2016; Lamie and Azmey, 2019). *In silico* and *in vivo* studies of *C. ternatea* anthocyanin as an anti-inflammatory candidate has not been explored yet, while comparison data from both studies will be needed for determining novel anti-inflammatory drug with fewer side effects, effective, and efficient. In this research, *in silico* study of anthocyanin and ternatin flavonoids as the potential binding ligands to the active site of inflammatory markers, including COX-1, COX-2, iNOS, and MPO using molecular docking analysis compared to that of ibuprofen as a native ligand for COX-1 was evaluated

2. Materials and methods

In silico study was conducted at DNA Technology and Bioinformatics Laboratory, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta (Indonesia). The automatic docking study was carried out using Protein-Ligand ANT System (PLANTS, version 4.6, Universität Konstanz, Germany) as implemented through the graphical user interface YASARA. Protein crystal structures of COX-1, COX-2, iNOS, and MPO complex (PDB codes: 1EQG, 3Q7D, 4UX6, and 5FIW; Figure 1) were retrieved from the RSCB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The PDB archives contained the drugs bound to the receptors. Ligand molecule structures of ibuprofen, anthocyanin flavonoid, and ternatin flavonoid (Figure 2) were obtained from MolView(http://molview.org/). All bound water and ligands were eliminated from the protein and the polar hydrogen using YASARA (http://www.yasara.org/).

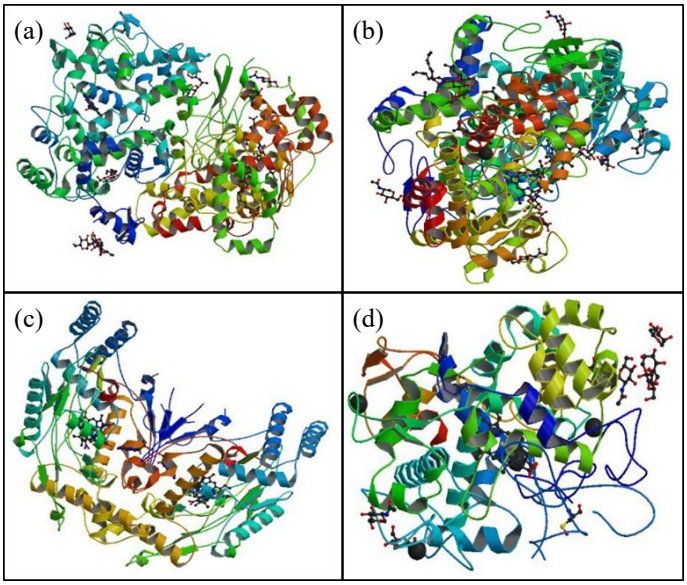

Figure 1. Protein crystal structures of COX-1(1EQG.pdb; a), COX-2 (3Q7D.pdb; b), iNOS (4UX6.pdb; c), and MPO (5FIW.pdb; d) as inflammatory markers for studying ligand-protein binding using molecular docking analysis. COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase

The other binding energy scoring was predicted by different algorithms using AutoDock Vina (version 1.1.2, The Scripps Research Institute, USA). All commands were put in one configuration file for parameter receptor and ligand. This configuration file was used as the guidance for running AutoDock Vina. For its input and output, AutoDock Vina used the PDBQT molecular structure file format. PDBQT files were generated (interactively or in batch mode) and viewed by using MGLTools (http://mgltools.scripps.edu/).

3. Results and discussion

In silico data (Figures 3-5) showed the docking results performed with PLANTS for all ligand compounds (ibuprofen, anthocyanin flavonoid, and ternatin flavonoid). The values of protein binding energy for all ligand compounds were listed in Table 1. Docking

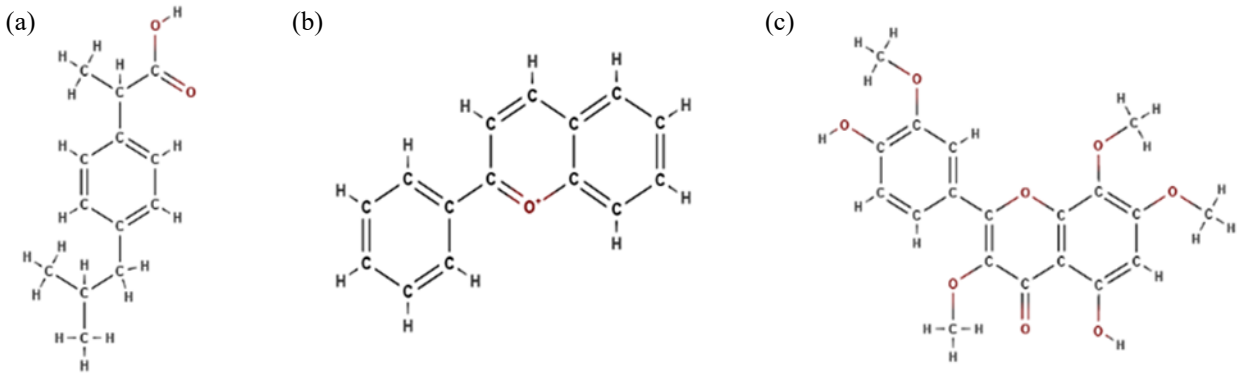

Figure 2. Ligand molecules including ibuprofen (a), anthocyanin (b), and ternatin (c). as inhibitors for studying ligand-protein binding using molecular docking analysis. COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase.

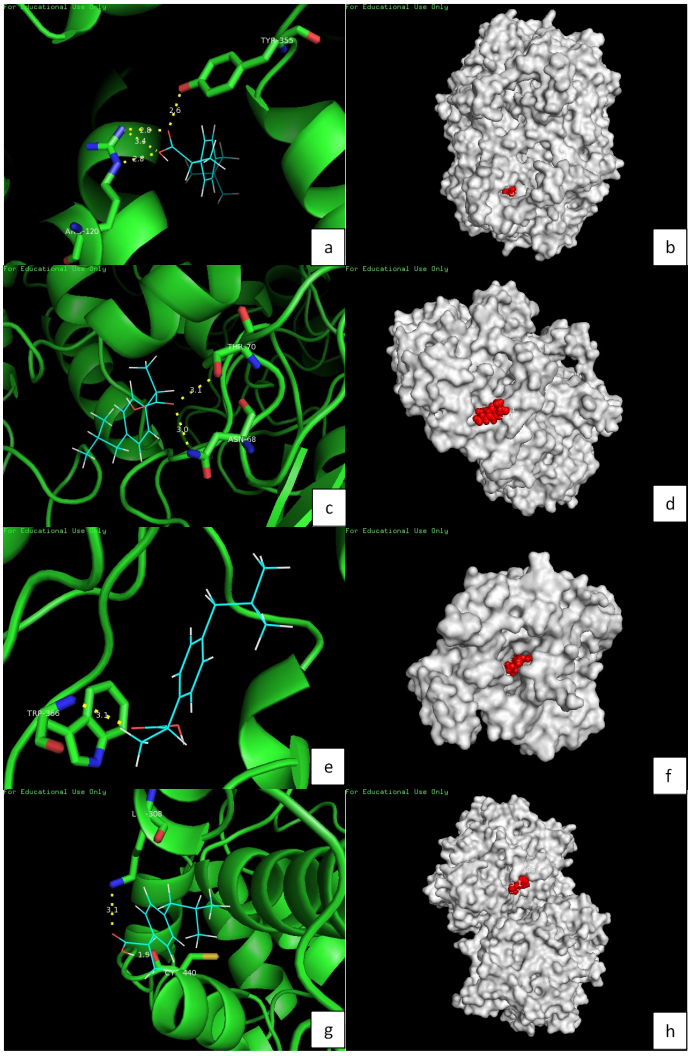

Figure 3. Bonding complex between ibuprofen with COX-1 (1EQG.pdb; a and b), COX-2 (3Q7D.pdb; c and d), iNOS (4UX6.pdb; e and f), and MPO (5FIW.pdb; g and h) using AutoDock Vina. COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase.

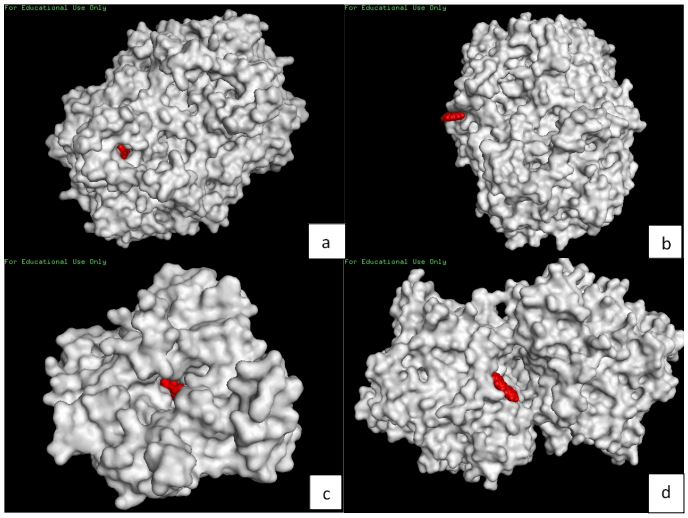

Figure 4. Bonding complex between anthocyanin with COX-1 (1EQG.pdb; a), COX-2 (3Q7D.pdb; b), iNOS (4UX6.pdb; c), and MPO (5FIW.pdb; d) using AutoDock Vina. COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase.

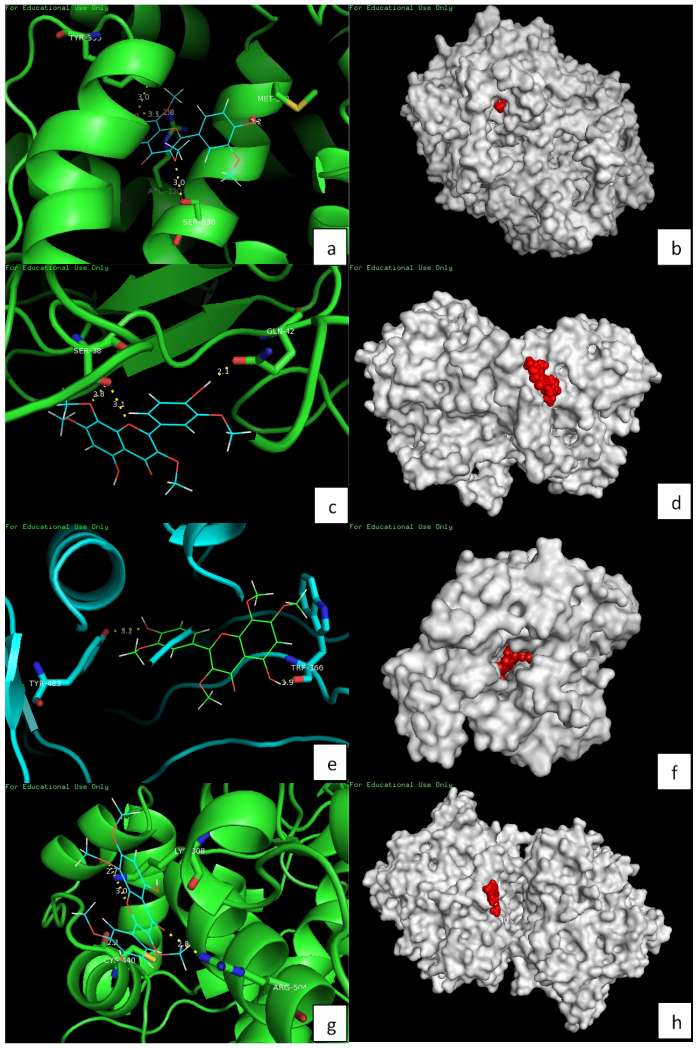

Figure 5. Bonding complex between ternatin with COX-1 (1EQG.pdb; a and b), COX-2 (3Q7D.pdb; c and d),iNOS (4UX6.pdb; e and f), and MPO (5FIW.pdb; g and h) using AutoDock Vina. COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase.

Table 1. Protein-ligand binding energy with PLANTS

Compound	Binding energy (kcal/mol)			
	COX-1	COX-2	iNOS	MPO
Ibuprofen	-80.60	-50.71	-81.95	-54.35
Anthocyanin	-78.47	-55.62	-93.05	-52.56
Ternatin	-56.16	-57.23	-95.54	-52.42

COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase

Table 2. Protein-ligand binding energy with AutoDock Vina

Compound	Binding energy (kcal/mol)			
	COX-1	COX-2	iNOS	MPO
Ibuprofen	-7.90	-6.80	-7.50	-6.00
Anthocyanin	-9.10	-7.90	-8.30	-6.90
Ternatin	-7.50	-7.70	-7.70	-7.00

COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase

analysis was performed by calculating binding energy values among ligand compounds and proteins regulating inflammatory responses (COX-1, COX-2, MPO, and iNOS). PLANTS was used to visualize docking among ibuprofen with COX-1 and COX-2. Ibuprofen was found to be a native ligand for COX-1. Our results also indicated that ibuprofen effectively bound to the active site of COX-1 with minimum binding energy (ΔG_b = -80.60 kcal/mol) but did not bind to the active site of COX-2 (ΔG_b = -50.71 kcal/mol).

Table 1 shows that ibuprofen was a potent inhibitor for COX-1, as the binding energy indicated its binding potential to the active site of COX-1. Ternatin was a potential inhibitor for COX-2, in which the binding site for this protein is long, slender with a hydrophobic channel extending from the membrane-binding region. A second cavity branched off from the main channel led to the cyclogenase active site was observed in COX-2, which bound greatly to ternatin. Anthocyanin and ternatin were bound into the active site of COX-1.

Based on PLANTS analysis (Table 1), anthocyanin had similar minimum binding energy (ΔG_b = -78.47 kcal/mol) with that of ibuprofen. Among the ligand compounds, lower COX-2 binding energy for ternatin (ΔG_b = -57.23 kcal/mol with PLANTS) compared to those of anthocyanin and ibuprofen (ΔG_b = -55.62 kcal/mol, ΔG_b = -50.71 kcal/mol). These data showed COX-1 bound effectively to small compounds, while COX-2 bound to larger compounds due to the size of its active site.

All ligand compounds had the ability to bind the active site of both COX-1 and COX-2 (Figures 3-5). The 2D-images of binding mode between each compound and the target proteins were demonstrated. The active pocket of COX-1 interacted with ibuprofen via Tyr-355 and Arg-120. COX-1 interaction with ternatin was mediated by Tyr-355, Arg-120, Ser-530, and Met-522. COX-2 showed interaction with ibuprofen via Thr-70 and Asn-68. COX-2 interaction with ternatin was shown at Ser-38 and Gln-42. The binding site of iNOS interacted with ibuprofen through Trp-366, and with ternatin at Trp-366 and Tyr-483. The MPO showed interaction with ibuprofen through Lys-308 and Cys-440, and interaction with ternatin via Lys-308, Cys-440, and Arg-504. The most substantial differences between these three inhibitors were found in the substituents on the phenyl ring which lie at the end of the molecule from the carboxylic acid group.

Docking studies based on AutoDock Vina showed that the three ligand compounds showed various binding energies (Table 2). Anthocyanin was able to inhibit COX -1, COX-2, and iNOS; therefore, it showed the highest potential for anti-inflammatory efficacy. However, anthocyanin binding to MPO was less effective. Anthocyanin and ternatin could effectively bind to the active site of COX-1. Based on PLANTS and AutoDock Vina analysis, values for binding energy and optimum binding energy for the active site of COX-1 were variable.

PLANTS results demonstrated that ibuprofen was the best compound for binding to the active site of COX-1, while AutoDock Vina results showed that anthocyanin was the best. This might be due to the fact that both software used different algorithms to calculate binding energy at protein active sites. All tested ligands (ibuprofen, anthocyanin, and ternatin) bound COX-2 active site, which was located at the apex of a long narrow hydrophobic channel extending from the membrane-binding surface to the center of protein (Figures 3-5). It should be noted that ibuprofen contains an isobutyl substituent on its phenyl ring, rather than a second aromatic group, and furthermore, this compound is not halogenated.

In the present study, *in silico* modeling based on ligand-protein binding energy was done. The *in-silico* data generated through PLANTS and AutoDock Vina demonstrated that ibuprofen, anthocyanin, and ternatin bound to COX-1 and COX-2 active sites (Figures 3-5 and Tables 1-2). These three compounds shared structural similarity, in which one or more phenyl rings are present in all of them. Compound size affects minimum binding energy for binding to the active site of the target protein (Tables 1 and 2). Docking poses generated by the PLANTS and AutoDock Vina can be directly loaded into PyMOL. Moreover, docking poses resulted in meta-information, i.e. the docking score for structure analysis of score relationships. All docking poses were ranked according to their docking scores, in which lower score indicated lower binding energy. Structure-based docking methods rank molecules *via* a scoring function that is calculated based on their inner methods (Yang *et al*., 2019). In addition, the previous study also showed that AutoDock Vina was a more efficient option for stimulating protein docking with macrolides and analogues of intermediate size ligand compared to other docking programs, such as Glide 6.6, AutoDock 4.2 and DOCK 6.5 (Castro-Alvarez *et al.,* 2017).

Both PLANTS and AutoDock Vina showed consistent molecular docking results for ibuprofen and COX-1, as well as for its competitors (anthocyanin and ternatin flavonoids) and its rapidly reversible mechanism for COX inhibition. Mechanism of inhibition by ibuprofen was observed for COX-1 and COX-2,

involving ligand binding to both proteins near the solvent -accessible opening of the hydrophobic channel, followed by a fast translocation of the inhibitor along the length of the channel, all of which associated with the COX active site. These results showed that ibuprofen binding orientation to COX-1 and COX-2 was similar. The carboxyl group of ibuprofen formed the hydrogen bond with Arg-120 and Tyr-355 at the COX-1 active site. Our results are in line with the previous study by Viegas *et al*. (2011), who conducted crystallography analysis of COX-1 and ibuprofen binding analysis using saturation transfer difference NMR (STD-NMR).

To date, there has yet been model for ibuprofen binding to COX-2 or anthocyanin and ternatin flavonoids binding to COX-1, COX-2, iNOS, and MPO. Interestingly, based on our *in silico* data generated through PLANTS (Figures 3-5), we were able to propose binding orientation and energy for ibuprofen binding to COX-1. This is also supported by the consistency of phenyl ring in ibuprofen for the docking arrangement and similarities among the positioning of aromatic ring in anthocyanin and ternatin with that of the phenyl ring in ibuprofen.

4. Conclusion

In silico analysis indicated that molecular docking of ternatin flavonoid bound effectively to the active site of most inflammatory proteins, including COX-1, COX-2, and iNOS, and MPO. Based on its binding energy, anthocyanin flavonoid was able to inhibit COX-1, COX-2, and iNOS. These data suggest that anthocyanin and ternatin flavonoids may act as potential inflammatory inhibitors as well as ibuprofen for treatment of inflammatory-related diseases.

Conflict of Interest

The authors declare no conflict interest.

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