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# Bioactive Compounds Analysis of *Averrhoa bilimbi* L. as Inhibitor of Cyclooxygenase-2 Enzyme Using in silico Approach

# (Analisis Senyawa Bioaktif *Averrhoa bilimbi* L. sebagai Penghambat Enzim Siklooksigenase-2 Menggunakan Pendekatan *in silico*)

ESTI MULATSARI[1*], YATI SUMIYATI[1], WARNI[1]

[1] **Faculty of Pharmacy, Universitas Pancasila, South Jakarta, DKI Jakarta, 12640, Indonesia**

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**Abstract:** *Averrhoa bilimbi* L. is generally used as a food flavor enhancer and traditional medicine to treat inflammation, cancer sores, cough, fever, gout, rectal bleeding, and hemorrhoids. In vivo and in vitro studies on *Averrhoa bilimbi* L. have shown anti-inflammatory activity, but the active compounds that play a role in anti-inflammatory activity have not been reported. This study aimed to analyze sixty-four (64) bioactive compounds in the *Averrhoa bilimbi* L. plant as cyclooxygenase-2 (COX-2) enzyme inhibitors using in silico approach and predict the pharmacokinetic and toxicological profiles of each compound. The cyclooxygenase-2 enzyme is an enzyme that plays a role in the inflammatory process by converting arachidonic acid into prostaglandin. Increased prostaglandins will cause inflammation. The research method used molecular docking with the application of YASARA, PLANTS, Marvinsketch, Pymol, visualization with PLIP and prediction of ADMET with pkCSM. Control compound used celecoxib. The results showed that there were 13 test compounds that were predicted to have better COX-2 inhibitor activity than celecoxib with good pharmacokinetic properties. Erucic acid has the best pharmacokinetic and toxicity profile. Erucic acid has the potential to be developed as a cyclooxygenase-2 enzyme inhibitor drug.

**Keywords:** Anti-inflamation, *Averrhoa bilimbi* L., cyclooxygenase-2, in silico

**Abstrak:** Belimbing wuluh (*Averrhoa bilimbi* L.*)* pada umumnya digunakan sebagai penambah cita rasa makanan dan obat tradisional untuk mengobati peradangan, sariawan, batuk, demam, encok, perdarahan pada rektum dan wasir. Penelitian secara *in vivo* dan *in vitro* terhadap belimbing wuluh menunjukkan adanya aktivitas antiinflamasi, namun belum dilaporkan senyawa aktif yang berperan dalam aktivitas antiinflamasi tersebut. Penelitian ini bertujuan untuk menganalisis enam puluh empat (64) senyawa bioaktif dari belimbing wuluh (*Averrhoa bilimbi* L.) sebagai inhibitor enzim siklooksigenase-2 secara *in silico* dan memprediksi profil farmakokinetika dan toksisitas masing-masing senyawa. Enzim siklooksigenase-2 merupakan enzim berperan dalam proses inflamasi dengan mengubah asam arakidonat menjadi prostaglandin. Prostaglandin yang meningkat akan menyebabkan inflamasi. Metode penelitian yang digunakan ialah *molecular docking* dengan aplikasi YASARA, PLANTS, Marvinsketch, Pymol, visualisasi dengan PLIP dan prediksi ADMET dengan pkCSM. Senyawa pembanding yang digunakan celecoxib. Hasil penelitian menunjukan ada 13 senyawa uji yang diprediksi memiliki aktivitas sebagai penghambat COX-2 yang lebih baik dibandingkan celecoxib dengan sifat farmakokinetika yang baik. Asam erukat memiliki profil farmakokinetika dan toksisitas yang baik. Asam erukat berpotensi untuk dikembangkan sebagai obat inhibitor enzim siklooksigenase-2.

**Kata kunci:** Anti-inflamasi, *Averrhoa bilimbi* L., *in silico*, siklooksigenase-2

*Corresponding author
e-mail: esti.mulatsari@univpancasila.ac.id

## INTRODUCTION

Traditional medicine is increasingly being used in medicine in both developing and developed countries. The World Health Organization (WHO) has also recommended the use of traditional medicine in maintaining health, preventing and treating disease[1].

*Averrhoa bilimbi* L., is a plant that is used by Indonesian people as a vegetable and is commonly used in cooking to add to the taste of food. *Averrhoa bilimbi* L. is easy to cultivate and easy to find in all parts of Indonesia[2,3]. Traditionally the fruit and leaves of *Averrhoa bilimbi* L. are often used by the community to treat inflammation, canker sores, coughs, fever, gout, rectal bleeding and hemorrhoids[4,5].

Anti-inflammatory activity in *Averrhoa bilimbi* L. has been tested in vitro using ethanol extract of *Averrhoa bilimbi* L., leaves suspended in bovine red blood cell suspension, centrifuged then the supernatant obtained was measured at a wavelength of 560 nm with a UV Vis spectrophotometer, the results showed the presence of anti-inflammatory activity based on the percentage of hemolysis inhibition of 91,18%[6]. *Averrhoa bilimbi* L. extract in mice with ulcer colitis at doses of 50 mg/kg and 100 mg/kg, based on histopathology of the colonic mucosa showed reduced cell damage, inflammation, and edema in the wound to level 2 compared to controls (level 9)[7]. Another research conducted have tested the anti-inflammatory effect of methanol extract from *Averrhoa bilimbi* L. leaves in Swiss albino rats at a test dose of 200 mg/kg BW giving the same anti-inflammatory effect as controls[8]. From several research results that have been reported, it has not been reported which metabolite compounds provide anti-inflammatory activity and how the anti-inflammatory mechanism works.

Inflammation is a normal response of the body to infection and tissue injury. Factors that can cause inflammation can come from endogenous factors and exogenous factors. The main causative factors are trauma, injury, surgery, infection, extreme heat and cold, and the body's immune response. The cyclooxygenase enzyme is one of the enzymes that plays a role in inflammation by converting arachidonic acid into prostaglandins resulting in inflammation and pain[7,9]. The presence of a virus in the body can induce the cyclooxygenase-2 enzyme so that the synthesis of prostaglandins increases[10]. Therefore the cyclooxygenase-2 enzyme can be used as a target in the search for better anti-inflammatory drugs. In this study an analysis of the inhibition of the bioactive compounds of *Averrhoa bilimbi* L., on the cyclooxygenase-2 enzyme will be carried out in order to obtain bioactive compounds that have inhibitory activity of these enzymes which can be carried out in silico.

## MATERIALS AND METHODS

**MATERIALS.** The 2D and 3D structures of the bioactive compounds contained in *Averrhoa bilimbi* L., leaves include: cucumerin A, afzelechin 3-O-alpha-L-rhamnopyranoside, ethyl 3-(N-butylacetamido) propionate, elaeokanine C, 2-ethyl-dodecanoic acid, isoavocadienofuran, (5α,8β,9β)-5,9-Epoxy-3,6-megastigmadien-8-ol, diglycidyl resorcinol ether, 19-hydroxycinnzeylanol 19-glucoside, xestoaminol C, phytosphingosin, 2-hydroxyhexadecanoic acid, pentadecanal, anapheline, palmitic amide, tetradecylamin, pentadecanoyl-EA, codonopsine, enigmol, 7-hexadecen-1-ol, (Z)-2-Ami727no-1-hydroxyoctadek-4-en-3-one, dihydroceramide C2, 6-hydroxysphingosine, methyl 8 -[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate, dehydrophytosphingosin, 14-methyl-8-hexadecen-1-ol, nonadecanal, oleoyl ethanolamide, linoleamide[11]. 2D and 3D structures of bioactive compounds in fruit of *Averrhoa bilimbi* L., include: hexadecanoic acid, squalene, erucic acid, oleic acid, chimanine D, 5-hydroxymethyl furfural, mannitol, desulphosinigrin, methyl pyroglutamate, trans-resveratrol, umbelliferon, salicylic acid, methyl salicylate, dihydromyricetin, eriocitrin, boswellic acid, hydroxy aristolic acid, cinnamaldehyde, benzyl cinnamate, lactone hydroxy citric acid, benzyl alcohol, phenethylamine, leaf alcohol, caffeoylmalic acid, citric acid, tartaric acid, ascorbic acid, xylose, tyrosine, phenelzine, benzeneacetamide, 2,4-di-tert-butylphenol, ɤ-stearolactone, 1-hentetracontanol, methyl ricinoleate[12–14]. The crystal structure of the cyclooxygenase-2 (COX-2) enzyme with the code 6COX from the Protein Data Bank (PDB) is taken from one structure with the best validation results[15]. The 2-dimensional and 3-dimensional structures of the comparator compound, namely celecoxib[16].

**Tools.** Hardwares: Laptop Asus A442U Intel Core i5-8250U CPU @1,6Ghz 1,8 GHz, Windows 10 Pro 64-bit operating system, x64 based processor RAM 4 GB.

Softwares: YASARA (http://www.yasara.org/viewdl.htm), MarvinSketch (http://www.chemaxon.com/marvin/download-user.html), Protein-Ligan ANT System (PLANTS) (http://www.tcd.uni-konstanz.de/index.php).

Webserver: Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) , Pubchem (http://pubchem.ncbi.nlm.nih.gov/), Pymol (https://pymol.org/2/), Protein-

Ligand Interaction Profiler (PLIP) (https://projects.biotec.tu-dresden.de/plip-web/plip), pkCSM (http://biosig.unimelb.edu.au/pkcsm/)

**METHODS. Selection of Test Compounds According to Lipinski Rules.** Enter the test compound file in the SMILES (simplified molecular input line entry system) format on the webserver http://biosig.unimelb.edu.au/pkcsm/. Then clicking run will get results for each parameter of the Lipinski rules.

**Cyclooxygenase-2 Enzyme Preparation.** The structure of the ligand-protein complex in (.pdb) format was obtained from the Protein Data Bank (PDB) downloaded from the site http://www.rcsb.org/. It was prepared again with the YASARA application so that 2 files were obtained, namely protein.mol2 and ref_ligand.mol2.

**Preparation of Native Ligand, Compound Compound Ligand and Test Compound Ligand.** Ligand preparation was carried out using MarvinSketch software at pH 7.4 and stored as ligand_2D.mrv. The conformational search was selected and the results of the conformational search were saved as ligands in the .mol2 formal file. This procedure was carried out for each native ligand, reference compound ligand and test compound ligand.

**Protein Validation (Redocking).** Validation was carried out using the PLANTS application with a 64-bit Windows operating system with the help of CMD. The prepared native ligand was then redocked on the protein crystal structure using the PLANTS program until a docking score was obtained and the best score was selected and then stored in a mol2 file. The RMSD value is calculated with the YASARA program. The parameters obtained are then used as the docking protocol.

**Docking Simulation.** Docking is done using the PLANTS application with a 64-bit Windows operating system via CMD command script. The ligand file for the control compound/test compound obtained from the preparation procedure was then docked using the PLANTS program. The docking score was obtained from the ligand of the control compound or the test compound. Then the docking scores of the test compounds were compared with the docking score values of the control compound.

**Visualization of Ligand and Protein Interaction.** A ligand-protein complex was prepared from a test compound that was predicted to have anti-inflammatory activity using the PyMOL application with the file type (*.pdb). Then visualized with PLIP. The visualization results are interpreted to determine the interactions of any amino acids that bind to the active site of the protein.

**ADMET Prediction (Absorption, Distribution, Metabolism, Excretion, Toxicity).** The SMILES file of the docking test compounds predicted to have anti-inflammatory activity was checked for absorption, distribution, metabolism, excretion, and toxicity predictions using the pkCSM webserver. The predicted value of ADMET is obtained.

## RESULTS AND DISCUSSION

**Protocol Validation with RMSD Determination.** 6COX is a crystal structure of the cyclooxygenase-2 (prostaglandin synthase-2) enzyme which forms a complex compound with SC-558 (COX-2 inhibitor). This receptor has a unique ligand, namely SC-558 or S58 (1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenylpyrazole) which acts as an inhibitor of the cyclooxygenase-2 enzyme[17,18].

The cyclooxygenase-2 enzyme is an enzyme that has a role in the inflammatory process by converting arachidonic acid into prostaglandins. Increased prostaglandins can cause inflammation and pain. Therefore this enzyme is considered as an important drug target in preventing inflammation or inflammation. Internal validation was carried out to see the ability of molecular docking simulations to create ligand poses in the target protein. This capability is illustrated by the RMSD (Root Mean Square Deviation) value based on the deviation of the docked pose distance compared to the 3D pose of the native ligand which has formed a complex with the protein (the original ligand structure of PDB) which is calculated and analyzed through the YASARA application. Internal validation of the docking method was carried out by redocking the original ligand from PDB. Internal validation was performed on 6COX. The redocking process was carried out 10 times. The RMSD value is close to zero, so the original ligand pose is more like to the redocked ligand. In general, the RMSD value limit used as a reference is less than equal to 2Å.

The receptor-coded PDB 6COX is a COX-2 enzyme that forms a complex compound with the SC-558 inhibitor (1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenylpyrazole). 6COX has bindingsite center coordinates x, y and z= 23.6651, 23.3126, 47.8265 and a bindingsite radius of 11.3323. The results of 10 repetitions for 6COX obtained the largest RMSD value of 1.6407 Å and the smallest RMSD value of 1.2184Å. The lowest ChemPLP score is -71.4326 with a RMSD value of 1.2184Å (Table 1 and Figure 1). This shows that the developed protocol can be accepted and used further for the docking process of the test compounds[19,20].

**Table 1. RMSD of Native Ligand 6COX.**

| Iteration | 6COX | |
|---|---|---|
| | Score ChemPLP | RMSD (Å) |
| 1 | -71,1883 | 1,6365 |
| 2 | -71,2484 | 1,6373 |
| 3 | **-71,4326** | **1,2184** |
| 4 | -70,6334 | 1,4321 |
| 5 | -70,7631 | 1,3415 |
| 6 | -70,7090 | 1,2216 |
| 7 | -71,1558 | 1,2199 |
| 8 | -70,9367 | 1,5368 |
| 9 | -71,2742 | 1,3354 |
| 10 | -70,6332 | 1,6407 |

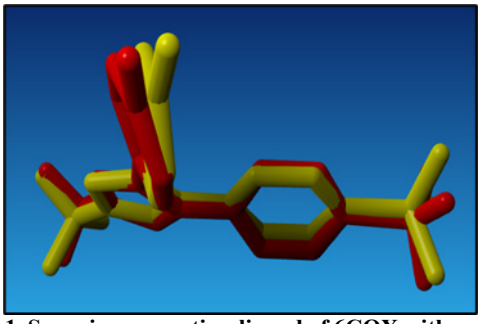

**Figure 1. Superimpose native ligand of 6COX with redocked ligand (red: native ligand of 6COX; yellow: redocked ligand)**

Bioactive compounds in *Averrhoa bilimbi* L. were analyzed as many as 64 compounds, namely 29 compounds in the leaves and 35 bioactive compounds in fruit[11–14]. The selection of test compounds based on the Lipinski rule aims to evaluate the physicochemical properties of these compounds when they cross cell membranes in the body. The Lipinski rule is a rule with the provisions that all constants are number 5 and their multiples consist of a molecular weight <500 Dalton, log P<5, hydrogen bond donors <5 and hydrogen bond acceptors <10. Lipinski's rule can predict the similarity of a compound with a drug so that it can predict the failure and success of an experiment. A test compound is declared not to comply with the Lipinski rules if there are 2 or 3 violations of these rules[21]. The results of the selection of 64 (sixty four) bioactive compounds from *Averrhoa bilimbi* L. obtained 4 bioactive compounds that did not meet Lipinski rules. These compounds have violations of 2 or more of the Lipinski rules, namely cucumerin A, 19-hydroxycinnzeylanol 19-glucoside, eriocitrin, 1-hentetracontanol as shown in Table 1. Meanwhile, as many as 60 bioactive compounds complying with the Lipinski rules are continued for the docking process with validated proteins.

**Docking Simulation Result.** There are 60 (sixty) bioactive compounds contained in *Averrhoa bilimbi* L. which comply with Lipinski rules by docking with 6COX validated protein. The test compounds were tested for their affinity for the 6COX receptor in silico using the molecular docking method using the PLANTS (Protein-Ligand ANT System) application.

Molecular docking, which is a method for designing or discovering new drugs using computers, has been widely used to predict whether a compound has pharmacological activity or not, and can be used for the development of compounds with better activity based on the interactions of compounds on the active sites of target receptors/proteins. Virtual screening with the molecular docking method aims to screen compounds to find out the best affinity through interactions between compounds or ligands on the cyclooxygenase-2 receptor. Good affinity is indicated by a lower ChemPLP value indicating a more stable bond. The ChemPLP score describes the value of the Gibbs free energy (ΔG) generated by the test compound for each conformation, where the smaller the score obtained the interaction of the ligand with the receptor will be more stable[22].

The docking simulation using the PLANTS application was carried out 10 times with 10 conformations. The best ChemPLP score from the docking results was chosen for the conformation that has the smallest ChemPLP score. The test compound that has a greater (more positive) ChemPLP score than the reference compound shows a weak affinity for the receptor binding site, whereas if the ChemPLP score obtained is more negative than the reference compound, it indicates that the affinity of the tested compound is stronger for the receptor binding site[22].

The results of the docking simulation performed on 60 test compounds at the 6COX receptor showed that 26 test compounds were more negative than celecoxib. ChemPLP of comparator compound Celecoxib was -85.7743. The 26 test compounds predicted to have the same or more active activity than the control compounds were isoavocadienofuran, xestoaminol C, phytosphingosine, 2-hydroxyhexadecanoic acid, pentadecanal, amide palmitate, pentadecanoyl-EA, enigmol, 7-hexadecen-1-ol, (Z) -2-amino-1-hydroxyoctadek-4-en-3-one, dihydroceramide C2, 6-hydroxysphingosine, methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate , dehydrophytosphingosine, 14-methyl-8-hexadecen-1-ol, nonadecanal, oleoyl ethanolamide, linoleamide, hexadecanoic acid, squalene, erucic acid, oleic acid, benzyl cinnamic, caffeoylmaleic acid, ɤ-stearolactone, methyl ricinoleate (Table 2). The test compound that had the most negative score was squalene with a

**Table 2. ChemPLP value, pharmacokinetic properties and toxicity of test compounds predicted to have COX-2 inhibitor activity.**

| NO | Test Compounds | CID | ChemPLP | Absorption | Distribution | | | Metabolisme | | | | Excretion | Toxicity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Intestinal Absorption (%) | VDss (L/kg) | Fraction Unbound | BBB Permeability (log BB) | CYP2D6 substrat | CYP2D6 inhibitor | CYP3A4 substrat | CYP3A4 inhibitor | Total Clearence (ml/min/kg) | LD50 (g/kg) | Hepatotoxic |
| 1 | Isoavocadienofuran | 90471631 | -88.8745 | 93.109 | 6.18 | 0.059 | 0.830 | Yes | No | Yes | No | 71.45 | 479.229 | No |
| 2 | Xestoaminol C | 14756407 | -90.1197 | 90.372 | 3.38 | 0.381 | -0.217 | No | No | No | No | 18.32 | 473.711 | No |
| 3 | Phytosphingosin | 122121 | -99.4931 | 94.240 | 0.49 | 0.432 | -1.359 | No | Yes | No | No | 26.92 | 565.803 | No |
| 4 | Asam 2-hidroksihexadekanoat | 92836 | -89.9313 | 90.023 | 0.20 | 0.195 | 0.002 | No | No | No | No | 67.92 | 373.488 | No |
| 5 | Pentadekanal | 17697 | -88.8767 | 92.676 | 2.99 | 0.115 | 0.800 | No | No | Yes | No | 61.94 | 339.374 | No |
| 6 | Amida palmitat | 69421 | -93.7585 | 90.399 | 2.08 | 0.117 | -0.332 | No | No | Yes | No | 68.71 | 460.303 | No |
| 7 | Pentadecanoyl-EA | 14455157 | -93.9886 | 90.546 | 1.43 | 0.196 | -0.241 | No | No | No | No | 87.30 | 505.853 | No |
| 8 | Enigmol | 11415391 | -98.9669 | 90.891 | 1.98 | 0.297 | -0.452 | No | Yes | Yes | No | 24.89 | 542.718 | No |
| 9 | 7-hexadecen-1-ol | 5352281 | -91.3186 | 90.310 | 2.81 | 0.110 | 0.788 | No | No | Yes | No | 82.04 | 372.651 | No |
| 10 | (Z)-2-amino-1-hidroksioktadek-4-en-3-on | 5280901 | -95.3988 | 91.121 | 1.36 | 0.309 | -0.407 | No | Yes | No | No | 23.77 | 629.468 | No |
| 11 | Dihidroceramide C2 | 6610273 | -103.445 | 91.049 | 0.56 | 0.187 | -0.950 | No | Yes | Yes | No | 114.02 | 614.937 | No |
| 12 | 6-hidroksisphingosine | 12991068 | -99.6283 | 95.174 | 0.49 | 0.446 | -1.302 | No | Yes | No | No | 18.24 | 554.947 | No |
| 13 | Metil 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-oktanoat | 5282975 | -100.640 | 97.004 | 0.40 | 0.250 | -0.472 | No | No | Yes | No | 53.09 | 541.010 | No |
| 14 | Dehidrofitosphingosin | 14757418 | -100.699 | 94.746 | 0.47 | 0.437 | -1.299 | No | Yes | No | No | 23.12 | 557.786 | No |
| 15 | 14-metil-8-hexadecen-1-ol | 5283286 | -90.5708 | 90.923 | 2.99 | 0.079 | 0.795 | No | No | Yes | No | 76.56 | 401.013 | No |
| 16 | Nonadekanal | 176926 | -95.0208 | 91.302 | 2.92 | 0.018 | 0.875 | No | No | Yes | No | 85.70 | 440.983 | No |
| 17 | Oleoyl ethanolamida | 5283454 | -101.783 | 90.021 | 1.26 | 0.113 | -0.340 | No | No | Yes | No | 123.59 | 593.116 | No |
| 18 | Linoleamida | 6435901 | -93.8604 | 90.724 | 1.80 | 0.068 | -0.363 | No | No | Yes | No | 102.33 | 499.116 | No |
| 19 | Asam hexadekanoat | 985 | -90.3848 | 92.004 | 0.29 | 0.101 | -0.111 | No | No | Yes | No | 57.95 | 369.245 | No |
| 20 | Squalen | 638072 | -110.862 | 90.341 | 2.58 | 0.000 | 0.981 | No | No | Yes | No | 61.80 | 759.011 | No |
| 21 | Asam erukat | 5281116 | -102.967 | 90.449 | 0.26 | 0.007 | -0.337 | No | No | Yes | No | 103.75 | 467.904 | No |
| 22 | Asam oleat | 445639 | -97.1213 | 91.823 | 0.28 | 0.052 | -0.168 | No | No | Yes | No | 76.56 | 400.246 | No |
| 23 | Benzil sinnamat | 5273469 | -87.7086 | 95.792 | 1.10 | 0.003 | 0.307 | No | No | Yes | No | 5.78 | 434.861 | No |
| 24 | Asam caffeoylmalat | 6124299 | -92.1159 | 14.637 | 0.17 | 0.444 | -1.170 | No | No | No | No | 2.87 | 646.670 | No |
| 25 | ɤ-stearolacton | 10396 | -92.7819 | 92.415 | 2.86 | 0.062 | 0.745 | No | No | Yes | No | 43.95 | 481.594 | No |
| 26 | Metil risinoleat | 5354133 | -98.2141 | 90.690 | 1.26 | 0.114 | 0.101 | No | No | Yes | No | 101.16 | 494.047 | No |

Note: *ChemPLP Celecoxib : -85.7743

ChemPLP score of -110.862. This shows that squalene has a better affinity for the 6COX receptor and has a more stable bond compared to other test compounds.

**Interactions of Test Compounds with Receptors.** The interactions of amino acids with the ligands formed after the docking process were visualized using the Protein-Ligand Interaction Profiler (PLIP) application. This application is a webserver-based application which can be accessed free of charge via the site https://projects.biotec.tu-dresden.de/plip-web/plip by entering a file with *pdb format of the protein-test compound complex which is predicted to have activity. Evaluation of residue interactions (amino acids) aims to identify the interactions that occur between the ligand and the receptor. Interactions can be hydrogen bonds, hydrophobic interactions, π-stacking, halogen bonds and salt bridges[23].

Hydrogen bonds are bonds that occur between hydrogen atoms and highly electronegative atoms such as N, O, F. Hydrophobic interactions are interactions that occur between hydrophobic molecules. π-stacking is a non-covalent interaction between two aromatic rings. Salt bridge is a combination of hydrogen bonds and ionic bonds, and is the strongest but rare[23,24]. The interaction between the receptor and the native ligand of the PDB code 6COX is in the form of hydrophobic interactions, hydrogen bonds, π-cation interactions and halogen bonds. Hydrophobic interactions occur at the amino acid residues Val349, Leu352, Tyr355, Phe518, Val523, Ala527. There are 4 hydrogen bonds in the amino acid residues Gln192, Ser353A, Arg513, Phe518. π-cation interaction and halogen bonding at the Arg120 amino acid residue. The amino acids that play a role are valine (Val), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), alanine (Ala), glutamine (Gln), serine (Ser), arginine (Arg). The bond that occurs between the receptor and the native ligand (SC-558) in the PDB code 6COX is shown in Figure 2. The interaction of the tested compound with the

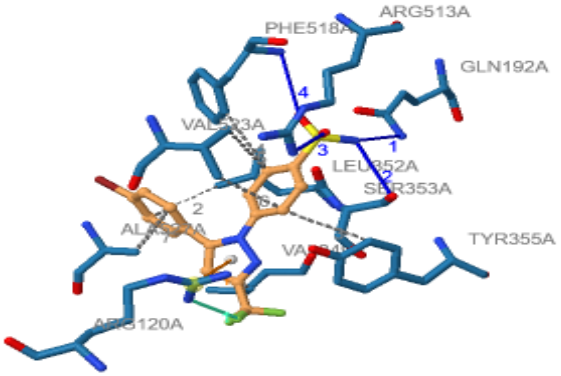


**Figure 2. Visualization of native ligand (SC-558 or 1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenylpyrazole) interactions with 6COX receptors (Blue line: hydrogen bonding. brown dashed line: hydrophobic interaction. orange line: π-cation interaction. and the green line: halogen bonds).**

6COX receptor includes hydrophobic interactions, hydrogen bonds and salt bridges. The amino acids that act as cyclooxygenase-2 enzyme inhibitors are the amino acids Arg120, Gln192, Val349, Leu352, Tyr355, Arg513, Phe518, Val523, Ala527 (Figure 3).

**Prediction of The Pharmacokinetic Profile and Toxicity of The Test Compounds.** The ADMET prediction was carried out on 26 test compounds which were predicted to have anti-inflammatory activity. The pharmacokinetic and toxicity profile of a drug is very important to assess its effectiveness. The pharmacokinetics observed included intestinal absorption, volume distribution steady state (VDss), unbound fraction, blood brain barrier permeability (BBB permeability), CYP450 represented by CYP2D6 and CYP3A4, total clearance, lethal dose (LD50) and hepatotoxicity.

The intestine is the main site for absorption of drugs given orally. A compound is said to have good absorption if it has an absorption value of >80% and poor absorption if <30%[25,26]. As many as 25 of the 26 tested compounds had absorption values ranging from 90.021% to 97.004%, only caffeoylmalic acid which had intestinal absorption values <30%. Drug distribution ability is influenced by 3 parameters, namely volume distribution steady state (VDSs), unbound fraction, blood brain barrier permeability (BBB permeability). VDss is said to be low if the value is 0.71 L/kg (log VDss<-0.15) and high if it is 2.81 L/kg (log VDss>0.45)[25]. There were 6 test compounds with a VDss value of >2.81 L/kg, namely isoavocadienofuran, xestoaminol C, pentadecanal, 14-methyl-8-hexadecen-1-ol, nonadecanal, ɤ-stearolactone so that these compounds have a high ability in uniform distribution.

Most of the drug in the plasma will be in equilibrium between the fraction that is not bound or bound to serum proteins. The efficacy of a drug is influenced by how much the drug binds to proteins in the blood. The more drug that is bound, the less efficient the drug can penetrate the cell membrane[25]. The unbound fraction has a value ranging from 0 to 0.446 and all compounds comply with this value except squalene.

A total of 22 bioactive compounds in *Averrhoa bilimbi* L are predicted to have anti-inflammatory activity with BW log values ranging from -0.95 to 0.981. This shows that the compound is able to penetrate the blood-brain barrier layer. Meanwhile, the 4 bioactive compounds, namely phytosphingosine, 6-hydroxysphingosine, dehydrophytosphingosine, and caffeoylmalic acid, had log BW values <-1, so it was predicted that these compounds would have poor distribution to the brain.

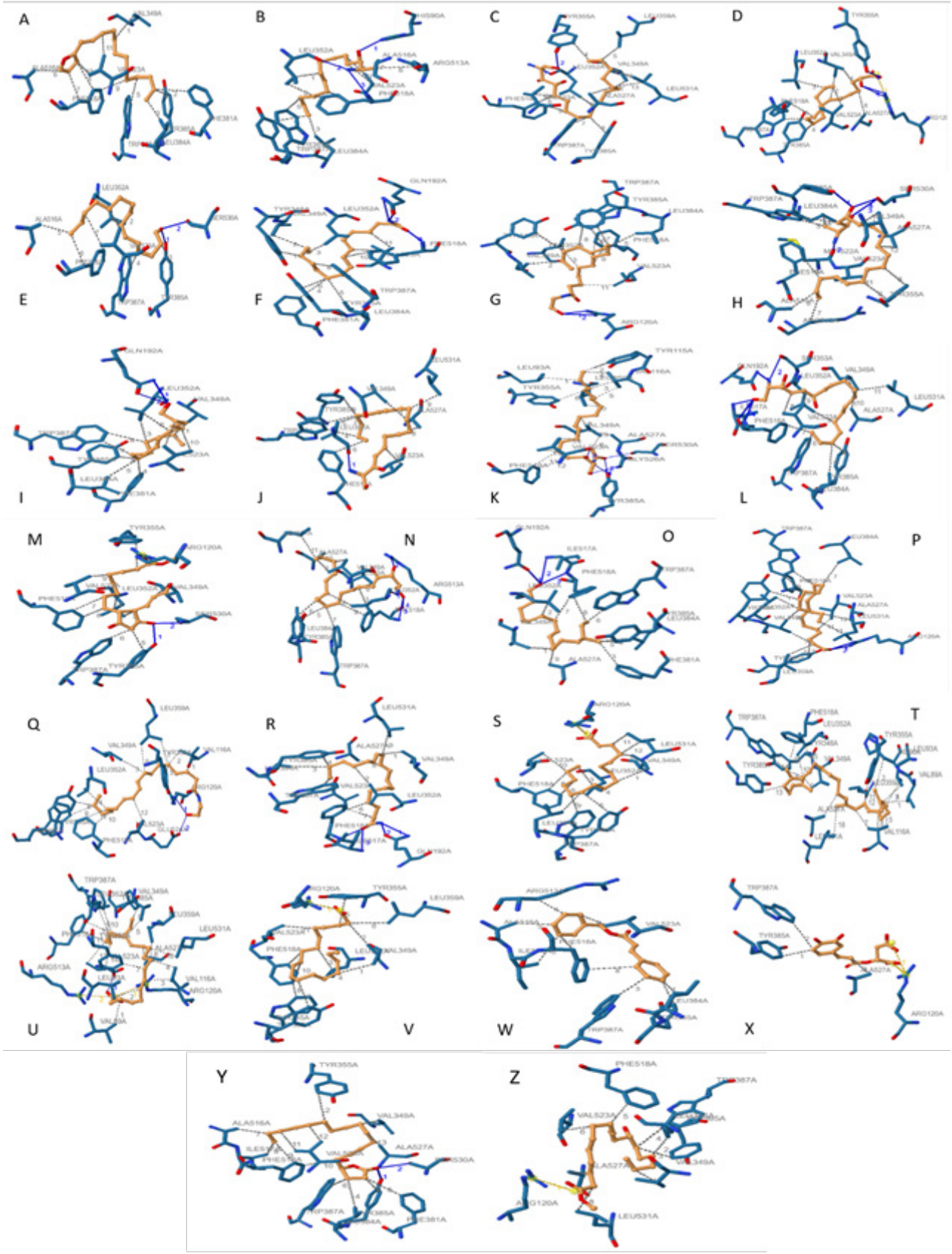


**Figure 3. Interaction of Test Compounds with 6COX Receptors**

**A) Isoavocadienofuran; B) Xestoaminol C; C) Phytosphingosine; D) 2-hydroxyhexadecanoic acid; E) Pentadecanal; F) Palmitic amide; G) Pentadecanoyl-EA; H) Enigmol; I) 7-Hexadecen-1-ol; J) (Z)-2-Amino-1-hydroxyoctadec-4-en-3-one. K) Dihydroceramide C2; L) 6-Hydroxysphingosine; M) Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate; N) Dehydrophytosphingosine; O) 14-methyl-8-hexadecen-1-ol; P) Nonadecanal; Q) Oleoyl ethanolamide; R) linoleamide; S) Hexadecanoic acid; T) Squalene; U) Erucic acid; V) Oleic acid; W) Benzyl cinnamate; X) Caffeoylmalic acid; Y) ɤ-stearolactone; Z) Methyl ricinoleate.**

The bioactive compound isoavocadienofuran in *Averrhoa bilimbi* L. is a CYP2D6 and CYP3A4 substrate. The bioactive phytosphingosine compound, enigmol, (Z)-2-Amino-1-hydroxyoctadec-4-en-3-one, dihydroceramide C2, 6-hydroxysphingosine, dehydrophytosphingosine is predicted to be a CYP2D6 inhibitor. Pentadecanal bioactive compounds, amide palmitate, enigmol, 7-hexadecen-1-ol, dihydroceramide C2, methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxocyclopentyl]-octanoate, 14-methyl -8-hexadecen-1-ol, nonadecanal, oleoyl ethanolamide, linoleamide, hexadecanoic acid, squalene, erucic acid, oleic acid, benzyl cinnamate, ɤ-stearolactone, methyl ricinoleate is predicted as a CYP3A4 substrate and there are no bioactive compounds in *Averrhoa bilimbi* L. that are as a CYP3A4 inhibitor. The bioactive compounds xestoaminol C, 2-hydroxyhexadecanoic acid, pentadecanoyl-EA, caffeoylmalic acid are predicted not to be substrates or inhibitors of CYP2D6 and CYP3A4[25].

The excretion process of drug compounds can be predicted by measuring the total clearance. Total clearance is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance. Total clearance is related to the bioavailability of the drug and to determine the dose level to achieve steady state concentrations. Total clearance is said to be low if <2 mL/min/kg, moderate 2-15 mL/min/kg, high 15-20 mL/min/kg and very high >20 mL/min/kg[27]. From Table 2 it can be seen that the total clearance value of bioactive compounds in *Averrhoa bilimbi* L ranges from 2.87 to 123.59 mL/min/kg. Based on the total clearance value, it can be predicted that the rate of excretion of these compounds is between moderate to very high.

The purpose of the toxicity test is to predict the toxic effect of a compound on a biological system and to obtain specific dose-response data from the tested compound. This data is used as information on the level of hazard of the test compound in the event of exposure to humans so that the dose of use can be adjusted[28]. In this study the toxicity test was carried out on the assessment of Lethal Dose 50 ($LD_{50}$) and hepatotoxicity. The $LD_{50}$ value is a standard measurement of acute toxicity that is used to assess the relative toxicity of a compound. $LD_{50}$ is the dose of the test compound that is given to cause the death of 50% of the test animal group[25]. Based on the Regulation of the Food and Drug Supervisory Agency of the Republic of Indonesia No. 7 of 2014, the $LD_{50}$ value is divided into 6 categories, namely very toxic (<1 mg/kg), toxic (1-50 mg), moderately toxic (50-500 mg), mildly toxic (500-5000 mg), practically non-toxic (5-15 g) and relatively harmless (>15 g). The $LD_{50}$ in test animals (rats) of the bioactive compounds of *Averrhoa bilimbi* L. which are predicted to have anti-inflammatory activity ranges from 339.374–759.011 g/kg which means that the bioactive compounds in *Averrhoa bilimbi* L are included in the toxicity level 6 meaning that they are relatively harmless.

Hepatotoxicity is a condition where compounds can induce liver function damage. A compound is classified as hepatotoxic if it has at least one pathological or physiological event related to impaired liver function[25]. In this study it was predicted that the bioactive compounds in *Averrhoa bilimbi* L would not cause hepatotoxicity.

Based on the prediction results of absorption, distribution, metabolism, excretion and toxicity carried out on 26 bioactive compounds in *Averrhoa bilimbi* L. which were predicted to have anti-inflammatory activity, it was found that 13 bioactive compounds in *Averrhoa bilimbi* L. had good pharmacokinetic profiles even though their excretion rates were very high (very high) and also does not cause toxicity. The bioactive compounds are isoavocadienofuran, pentadecanal, amide palmitate, enigmol, 7-hexadecen-1-ol, 14-methyl-8-hexadecen-1-ol, nonadecanal, oleoyl ethanolamide, linoleamide, erucic acid, benzyl cinnamate, ɤ-stearolactone, methyl ricinoleate. The ChemPLP score of the test compound was more negative than the reference compound, and erucic acid had a good pharmacokinetic and toxicity profile. This compound has the potential to be developed as an anti-inflammatory drug candidate. Erucic acid belongs to a group of long-chain unsaturated fatty acids which are composed of 22 carbon atoms with one double bond at the 13th carbon atom or omega 9. Another name for erucic acid is (Z)-13-dokosenoic acid. Based on Calder's 2012 study, it was stated that long-chain unsaturated fatty acids can affect the inflammatory process by reducing levels of arachidonic acid which is converted into prostaglandins[29]. Previous research showed that erucic acid was found to exert an inhibitory effect on pro-inflammatory mediators induced by viruses as well as interferons by inactivating the Nf-kB and p38 MAPK pathways thereby causing reduced transcriptional activity of interferon-stimulated gene factors 3. Thus reducing amplification pro-inflammatory response[30]. Therefore, erucic acid has the potential to be developed as an anti-inflammatory drug.

## CONCLUSION

Thirteen bioactive compounds in *Averrhoa bilimbi* L. that are predicted to have anti-inflammatory activity, have a good pharmacokinetic profile, and are not toxic include: isoavocadienofuran, pentadecanal,

amide palmitate, enigmol, 7-hexadecen-1-ol, 14-methyl-8-hexadecen-1 -ol, nonadecanal, oleoyl ethanolamide, linoleamide, erucic acid, benzyl cinnamate, ɤ-stearolactone, and methyl ricinoleate.

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