ANTI-INFLAMMATORY ACTIVITY OF STEM BARK DICHLOROMETHANE FRACTION *Syzygium samarangense* EXTRACT AS COX-2 INHIBITOR: A BIOINFORMATICS APPROACH

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Abstract

Syzygium samarangense is a plant that is rich in flavonoid compounds. Previous research revealed that the dichloromethane fraction from the stem bark extract contained four bioactive compounds, namely, pinocembrin, uvangoletin, stercurensin, and aurentiacin, which had good antioxidant activity. However, research on the potential of these four compounds as an anti-inflammatory has not been revealed. This study aims to continue previous research in revealing the potential of compounds in the dichloromethane fraction of *S. samarangense* extract as an anti-inflammatory agent *in silico* COX-2 inhibitors. This study uses *in silico* biocomputation, including drug-likeness analysis and molecular docking analysis using COX-2 protein and the control drug rofecoxib. The results showed that there are compounds that have the potential as anti-inflammatory compounds, namely pinocembrin. However, further studies, such as *in vitro* and *in vivo,* are still needed to reveal its potential as an anti-inflammatory agent.

Keywords*: anti-inflammatory, COX-2, Syzygium samarangense, in silico.*

Introduction

The body's response to pathogens and aggravations is inflammation, which can also be a protective response involving healthy cells, blood vessels, and molecular mediators (Ferrero-Miliani et al., 2007; Zhao et al., 2021). Inflammatory cells leukocytes, which include neutrophils, lymphocytes, and macrophages, are involved in this complex reaction (Abdulkhaleq et al., 2018). Inflammation can be caused by various mediators in the body. In a mechanism that may support inflammatory events, immunological protective cells target a pool of secreted mediators and various signaling molecules, such as histamine, prostaglandins, leukotrienes, oxygen and nitrogen-derived free radicals, and serotonin. (Anwikar & Bhitre, 2010). Vasoactive amines like histamine and serotonin, peptides (e.g., bradykinin), and eicosanoids are some chemical mediators produced (e.g., thromboxane, leukotrienes, and prostaglandins). One of the enzymes involved in the production of prostaglandins in the body is COX-2. COX-2 (Cyclooxygenase 2) is an enzyme that plays a role in the metabolism of chidonic acid in the production of prostaglandins (particularly PGE2 and PGI2) (Desai et al., 2018). With the overexpression of cyclooxygenase-2, prostaglandins also play a significant role in the pathogenesis of several cancer types, including breast, liver, and lung cancer (Zhang & Jordan, 2010). The production of prostaglandins from COX-2 will result in inflammation and neoplasia. Inflammation and neoplasia caused by COX-2 expression will

increase the risk of disease, especially the risk of cancer (Desai et al., 2018). Drugs commonly used in inflammation are nonsteroidal anti-inflammatory drugs (NSAIDs). One of the NSAIDs that has good effectiveness in overcoming inflammation and selectively inhibits COX-2 only is rofecoxib. Rofecoxib is a drug that inhibits COX-2 in the synthesis of prostaglandins that cause inflammation and has been approved by the FDA (Chen et al., 2008; FDA, 2005). But long-term use of NSAIDs also carries the risk of asthma, kidney failure, cardiovascular toxicity, and stomach ulcer side effects (Maseda & Ricciotti, 2020). So we need natural ingredients that have fewer side effects.

One of the natural ingredients with great potential is the plant Sygyzium samarangense. The stem bark of the plant has been investigated and reported in previous studies, where the dichloromethane fraction of the bark extract contains four compounds, namely pinocembrin, uvangoletin, stercurensin, and aurentiacin (Tukiran et al., 2021). Studies on the potential of these four compounds as anti-inflammatory have not been well reported. Therefore, research is needed to reveal the potential of these four compounds as anti-inflammatory agents. This study aimed to describe the potential of compounds in the dichloromethane fraction of the methanolic extract of the stem bark of *S. samarangense* as an anti-inflammatory agent of COX-2 inhibitors. The basic approach that can be taken is an *in silico* approach using molecular docking.

Research Methods

Sample preparation

The compounds found in the dichloromethane fraction of the stem bark extract of *S. samarangense*, including pinocembrin, uvangoletin, stercurensin, and aurentiacin (Tukiran et al., 2021), were the target substances used in this study. The control drug compound as an anti-inflammatory used in this study is the compound rofecoxib, a selective NSAID that inhibits COX2 activity and has been used as a chronic arthritis treatment drug and has been approved by the FDA (Cairns, 2007). All ligand samples were obtained from PubChem (pubchem.ncbi.nlm.gov). All ligand compounds were minimized using OpenBabel, which is in PyRx software, because this will allow the ligands to be flexible and then convert structure in file structure data format (.sdf) into protein databank format (.pdb) (Pradeepkiran et al., 2016). The protein receptor used in this study is COX-2 (1PXX), obtained from the RCSB database (rcsb.org). The protein was prepared using PyMOL to obtain a sterile protein and was ready to be analyzed using molecular docking.

Drug-likeness analysis

The dichloromethane fraction of *S. samarangense* stem bark was analyzed to determine its probability as a drug compound using Lipinski's five rule. Lipinski's five rules include a molecular weight ≤ 500 Daltons, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5, molar refractivity between 40–130, and lipophilicity (log P) less than 5 (Lipinski, 2004). Drug-likeness analysis was carried out using the Scfbio web server (scfbio-iitd.res.in/) (Jayaram et al., 2012).

Molecular docking and visualization

Molecular docking analysis was conducted to ascertain the interaction between ligand (bioactive compounds) and receptor proteins. Molecular docking was carried out to determine the binding affinity complex formed. AutoDock Vina 4.2 through PyRx software was used to perform molecular docking (Trott & Olson, 2010), COX-2 protein, which has been sterilized, is docked with ligand compound with dimensions X: 110.5566; Y: 90,7700; and Z: 140.0203 with coordinates X: 42.3638; Y: 33.8011; and

Z: 36.8530. Compounds with a lower binding affinity value than the control drug rofecoxib were interacted with and visualized in 2D and 3D to determine the position and type of interaction formed. Visualization was carried out using PyMOL software and Discovery Studio.

PASS-online prediction

Evaluation of potential compounds with anti-inflammatory activity was carried out by the PASSOnline (Prediction of Activity Spectra for Substances) web server. The results of these checks are defined by the probabilities Pa (possibility of activity) and Pi (possibility of inactivity), respectively, with values ranging between 0.000-1.000.(Lagunin et al., 2000). A potential compound's anti-inflammatory activity was predicted using PASSOnline when the Pa score was greater than 0.3. (moderate probability) (Rahmaningsih & Pujiastutik, 2019).

Results and Discussion

This study is a follow-up study on the potential bioactivity of the bark extract of *S. samarangense* in the medical world. The compound of the dichloromethane fraction of the bark extract of *S. samarangense* has been reported in the study of Tukiran et al. (2021) which previously included pinocembrin, uvangoletin, stercurensin, and aurentiacin were analyzed for their potential as anti-inflammatory agents. The ligand structure can be seen in Figure 1. The four compounds were analyzed for drug-likeness using Lipinski's five rules (RO5) to determine their potential as medicinal compounds. Lipinski's five rules include a molecular weight ≤ 500 Daltons, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5, molar refractivity between 40–130, and lipophilicity (log P) less than 5 (Lipinski, 2004). The analysis aims to select the four compounds in the molecular docking analysis. The results of the drug-likeness analysis (Table 1) indicate that the four compounds from the dichloromethane fraction have potential as medicinal compounds because they fulfill at least 3 of Lipinski's rules. The four compounds were analyzed using molecular docking.

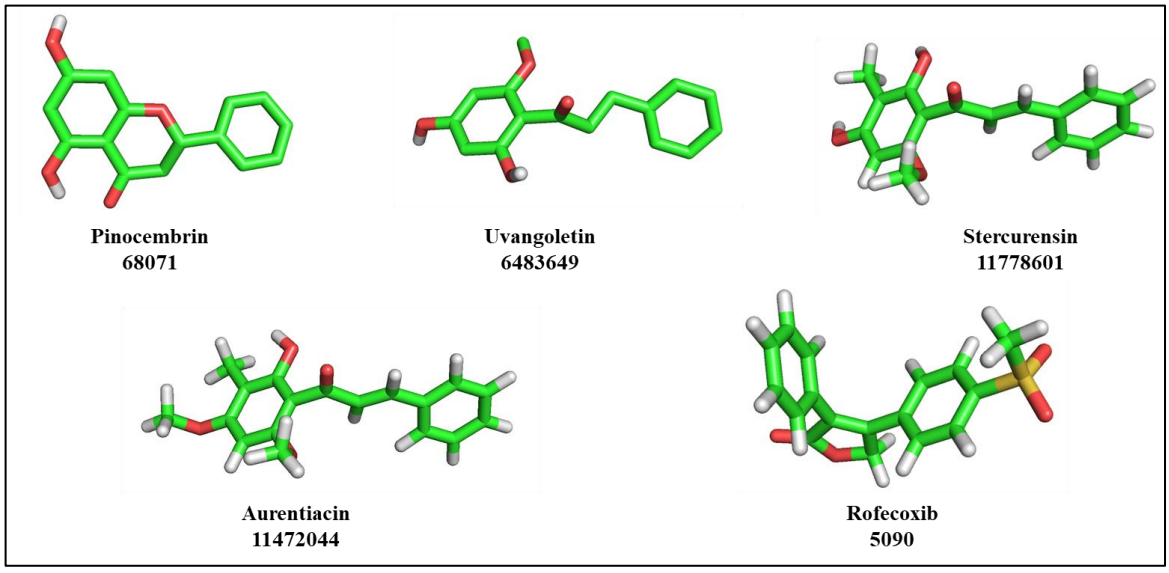


Figure 1. Molecular visualization of ligand structure

Molecular docking analysis aims to determine the potential of compounds as drugs with certain bioactivity by inhibiting target protein receptors. Prior to molecular docking analysis, ligands were minimized to obtain flexible ligands. When the ligand is flexible, a minimal (stable) binding affinity is expected to be

obtained (Pradeepkiran et al., 2016). In this study, the intended bioactivity is anti-inflammatory with the target protein is COX-2. The results of the molecular docking analysis (Table 1) showed that the four compounds interacted with COX-2 because of the negative binding affinity value. Because it has a lower binding affinity value than the control drug compound rofexocib, one compound, namely pinocembrin which was identified based on the molecular docking results as having the potential to be an anti-inflammatory COX-2 inhibitor. Binding affinity is the value of the stability of the complex between the protein-ligand formed (Muchtaridi et al., 2018). The more negative the binding affinity value of the complex, the more stable the stability of the complex. The more stable the formed complex, the more optimal the inhibitory activity (Jensen, 2017; Pires et al., 2018). The results showed that pinocembrin has a lower binding affinity value than rofecoxib which indicates that pinocembrin has a better potency than rofecoxib as an anti-inflammatory drug compound.

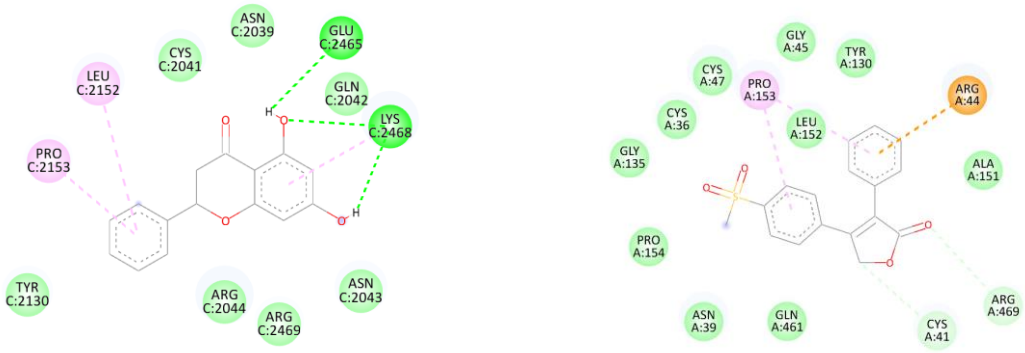
Table 1. The result of drug-likeness and molecular docking analysis

Compound	Drug-likeness Paramater					Binding Affinity (kcal/mol)
	MW[1]	AH[2]	DH[3]	MR[4]	Log P[5]	
Rofecoxib (Control)	-	-	-	-	-	-8.5
Pinocembrin	256.25	4	2	69.55	2.48	**-9.0**
Uvangoletin	272.30	3	2	76.47	2.92	-7.9
Stercurensin	284.31	3	2	81.75	3.20	-8.4
Aurentiacin	298.33	4	1	86.22	3.51	-7.7

Note : [1]MW = molecular weight $\leq$ 500 dalton; [2]AH= acceptor hyrdogen $\leq$ 10; [3]DH = donor hydrogen $\leq$ 5; [4]MR = molar refractivity 40 – 130; [5]Log P = lipophilicity $\leq$ 5

Pinocemberin was then visualized to determine the type and interactions formed. The visualization results (Figure 2) showed that the pinocembrin-COX2 complex was formed with 3 types of hydrogen bond interactions, hydrophobic bonds, and van der Waals interactions. Interactions that contribute more to the inhibitory activity of proteins are electrostatic bonds, hydrogen bonds, and hydrophobic bonds (Leelananda & Lindert, 2016; Young, 2009). Hydrogen bonds at positions Lys 2468 and Glu 2465; while the hydrophobic bonds at Leu 2152 and Pro 2153 positions. Hydrophobic interactions, van der Waals forces, electrostatic interactions, and hydrogen bonds all combine to form a complex in rofecoxib-COX2. An electrostatic bond is formed at position Arg 44; hydrogen bonds are formed at positions Cys 41 and Arg 469; then a hydrophobic bond is formed at Pro 153 position.

Based on the results of the analysis, it was found that the compound pinocembrin will inhibit COX-2 in prostaglandin synthesis so that the risk of inflammation caused by COX-2 can be inhibited. The COX-2 signaling pathway is initiated when chidonic acid induces COX-2 and produces PGE2 and PGI2 (prostaglandins) which cause inflammation and neoplasia. (Chen et al., 2008). The resulting inflammation and neoplasia will enhance other signaling pathways such as tumor/cancer cell invasion, angiogenesis, cell proliferation, macrophages, and synoviocytes. (Desai et al., 2018). When COX-2 is inhibited, the regulation of its signaling pathway can also be inhibited and the resulting effects will also be inhibited.



(**a**) (**b**)

Figure 2. Visualization interaction, (a) pinocembrin (b) rofecoxib

Evaluation of molecular docking results was carried out using PASSOnline to predict the potential anti-inflammatory activity of pinocembrin. The evaluation results in Table 2 show that pinocembrin has an activity probability (Pa) as anti-inflammatory of 0.677 and an inactive probability (Pi) of 0.019. In addition, the active probability value (Pa) as a cyclooxygenase substrate is 0.328 and the inactive probability (Pi) is 0.011. These two values represent that pinocembrin has potential as an anti-inflammatory with a Pa value > 0.3 or a moderate probability. If the Pa value is close to 1, there is an increase in anti-inflammatory activity (Kharisma et al., 2021; Rahmaningsih & Pujiastutik, 2019).

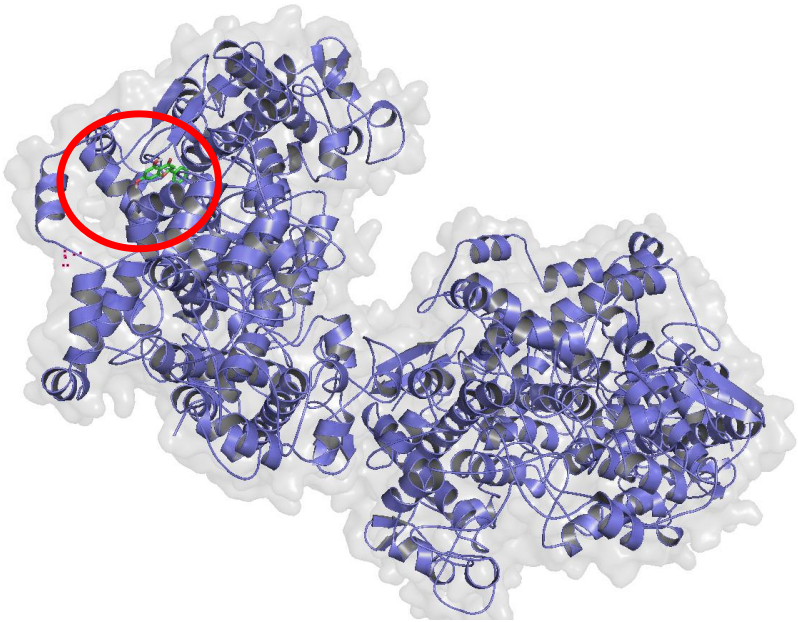


Figure 3. Surface area interaction of pinocembrin-COX2 complex

Table 2. The result of PASS-online prediction

Potential Compounds	Pa[a]	Pi[b]	Biological Activity
Pimocembrin	0,677	0.019	Antiinflammatory
	0.328	0.011	Cyclooxygenase substrate

Conclusions

The dichloromethane fraction identified pinocembrin as a potential compound for use as an anti-inflammatory agent by inhibiting COX-2 regulation, according to the results. Pinocembrin binds to COX-2 to prevent the production of prostaglandins, which are produced when COX-2 receptors process arachidonic acid and cause inflammation. With a low binding energy (9.0 kcal/mol) and a high activity probability score, the pinocembrin molecule complex binds. So it's possible that pinocembrin has anti-inflammatory properties.

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