Investigating the potency of bioactive compounds from *Ficus religiosa* as anti-inflammatory agent

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**Abstract** The previous studies explain that bioactive compounds from *Ficus religiosa* are known as an anti-inflammatory agent. In this research, we investigated the potency of bioactive compounds from *Ficus religiosa* by using molecular docking between eight bioactive compounds and the COX-2 receptor. The eight ligands were collected from www.pubchem.ncbi.nlm.nih.gov, while the receptor was taken from www.rcsb.org. The result of this study could be a reference for the research in the synthesis of the bioactive compound to minimize failure. The collected data was calculated through Autodock Vina embedded in MGL Tools 1.5.6, and these processes were performed using 100 runs of the Lamarckian Genetic Algorithm (LGA). The lowest energy of complexes was visualized by using Biovia Discovery Studio Visualizer. This result proved that 28-Isofucosterol-COX-2 had smaller binding energy compared to the reference ligand.

1. **Introduction**

Inflammation is a nonspecific immune system and biological response of the vascular system against stimuli such as bacteria, viruses, wounds, toxins, irritants, air pollutants and cell damages [1]. Leucocyte proteinase can cause tissue damages during inflammation which then produced inflammatory mediators such as neuropeptides, histamine, serotonin, potassium ion, arachidonic acid, and bradykinin. Macrophages play an important role in the inflammation mechanism by activating when the body is pathologically wounded and producing Reactive Oxygen Species (ROS). The macrophages will then attack the invading pathogen by releasing cytokine. This will trigger the release of a pro-inflammatory cytokine such as Tumor Necrosis Factor α (TNF α), Interleukins (IL-1β, IL-1β); and prostaglandin E2
The inflammation process can also be caused by physical symptoms such as reddish rash, uncomfortable pain and heat [3]. This can develop into acute inflammation which can further develop into chronic inflammation if not treated properly. Without proper treatment, the symptoms can further develop into fatal auto-immune and cellular damages diseases such as osteoarthritis, rheumatoid arthritis, gastric ulcers, Crohn’s disease, Metabolic Syndrome-associated Disorders, retinal neovascularization and cancer [4].

Use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) to inhibit the synthesis of prostaglandin is still been performed to present days even though long-term use of the drugs has been linked to adverse effect into the gastrointestinal system. In 1999 rofecoxib and celecoxib is commercially available as cyclooxygenase-2 (COX-2) receptor inhibitor, but further research showed that the compounds can cause an adverse effect on the cardiovascular system [5]. In present days, there is increasing interest in isolating bioactive compounds from plants as anti-inflammatory agents. Plants have been known to produced biologically active secondary metabolite compounds such as phenolics, saponins, terpenoids and alkaloids which can be potential COX-2 inhibitors [6]. One such plant which has been used in traditional medicine is Ficus religiosa [7–9].

There have been several in vivo and in vitro studies on the effect of the extract of Ficus religiosa. The previous study had reported that the fruit extract can be used to managed pain and inflammation [10], and the bark extract had been shown to be a potent inhibitor of the synthesis of histamine, serotonin, kinin, and prostaglandin [11]. However, due to the nature of the extract, there had not been many studies looking into the mechanism of inhibition and specific of the bioactive compounds. In this study, we used molecular docking methods to some compounds that have been isolated from Ficus religiosa in order to identify possible candidates as potential COX-2 inhibitors and thus potential anti-inflammatory agents.

2. Methods
In this study, we select 8 compounds (28-isofucosterol, bergapten, bergaptol, β-sitosterol, limonene, quercetin, serotonin, and sitosterol) isolated from Ficus religiosa [7] as the ligand, chain A of COX-2 protein (PDB ID: 5IKR) as the receptor, and mfenamic acid as reference ligand. The structure of the 8 compounds is retrieve from www.pubchem.ncbi.nih.gov and the receptor structure is retrieve from www.rcsb.org. The protein structure is prepared using Chimera 13.1 to isolate the desired chain. Molecular docking calculation is performed using AutoDock packages software in a cubic box of size 40 points with a spacing of 0.375 Å using 100 runs of Lamarckian Genetic Algorithm. The result of the calculation is then visualized using Biovia Discovery Studio Visualizer [12].

3. Results and discussion
The compounds selected from Ficus religiosa were used as ligands to be docked against the COX-2 receptor binding site to explore the affinity of the ligands with the said receptor. The molecular docking calculation results of the ligands were then compared to the reference ligand, Mefenamic Acid. Calculation results showed that Mefenamic Acid is docked into a COX-2 binding site with the binding energy of -7.59 kcal mol⁻¹ and hydrogen bonding with TYR385 and SER530 of COX-2. This result is consistent with the previous study and experimental structure of the COX-2-Mefenamic Acid complex retrieved from www.rcsb.org. The interaction and binding energy of all ligands shown in Table 1.
Table 1. Binding energy and amino acid residue the ligands form a hydrogen bond with COX-2 binding site.

| No | Compounds         | Binding Energy (Kcal mol⁻¹) | RMSD (Å) | Amino Acids                  |
|----|-------------------|-----------------------------|----------|------------------------------|
| 1  | 28-isofucosterol  | -11.55                      | 2.49     | -                            |
| 2  | Bergapten         | -6.94                       | 3.01     | TYR385                      |
| 3  | Bergaptol         | -6.55                       | 2.11     | VAL523                       |
| 4  | β-sitosterol      | -10.93                      | 1.82     | -                            |
| 5  | Limonene          | -5.88                       | 1.95     | -                            |
| 6  | Quercetin         | -6.79                       | 1.82     | ARG120, TYR385, SER530, TYR355, MET522 |
| 7  | Serotonin         | -5.31                       | 5.48     | SER530                       |
| 8  | Sitosterol        | -10.82                      | 4.7      | -                            |
| 9  | Mefenamic Acid    | (reference ligand)          | -7.59    | 0.58 TYR385, SER530          |

There are 3 ligands (28-isofucosterol, β-sitosterol, and sitosterol) - bind to the COX-2 binding site stronger than Mefenamic Acid. This can be observed from the fact that lower binding Mefenamic Acid had binding energy of -7.59 kcal mol⁻¹, while 28-isofucosterol, β-sitosterol, and sitosterol have a binding energy of -11.55, -10.93 and -10.82 kcal mol⁻¹ respectively (see Figure 1). The lower binding energy of the 3 ligands relative to Mefenamic Acid indicating that they bind more strongly to the COX-2 receptor and thus suggesting higher inhibition activity than Mefenamic Acid.

Sitosterol and β-sitosterol have comparable binding energy with a relative difference of approximately 1% suggesting that the two ligands should have comparable activities. The previous study reported that β-sitosterol was shown to significantly inhibit the expression of TNF-α-stimulated HAEC (Human Aortic Endothelial Cells) [13], inhibit inflammatory of rat ear edema [14] and inhibit transcription of NF-κB pathway. Sitosterol and β-sitosterol are phytosterol type compounds that also have been reported to have potential activity as anti-inflammatory agents through the inhibition of pro-inflammatory cytokine (IL-6 and TNF-α) [3]. TNF-α cytokine is produced by macrophages and T-Cells due to the stimuli, but overproduction of TNF-α cytokine can lead to chronic inflammation or in more severe cases, direct DNA damages [15]. Activation of TNRI (one of TNF-α receptor) will stimulate Mitogen-Activated Protein Kinase (MAPK), c-Jun N-terminal Kinase (JNK), and Nuclear Factor κ-light-chain enhancer of activated B cells (NF-κB) pathway which then would induce the expression of COX-2 receptor gene. Therefore, the COX-2 receptor metabolism had to be inhibited so that the enzyme cannot catalyze Arachidonic acid [15]. Based on the structural similarities, and the lower binding energy, it could be suggested that 28-isofucosterol is a potent anti-inflammatory agent.
Despite 28-isofucosterol, β-sitosterol, and sitosterol strong binding with the COX-2 receptor, none of the ligands formed hydrogen bonds with the TYR385 and SER530 which bonded with Mefenamic Acid, even though they interact weakly with said residue through van der Waals and/or hydrophobic (π-alkyl and π-σ) interaction. 3 other ligands though did form hydrogen bond with one or both amino acid residues. Bergapten bonded with TYR385, serotonin with SER530 and quercetin with both residues.

In Figure 2, it can be seen the comparison between 28-isofucosterol and quercetin interaction with the COX-2 binding site. While 28-isofucosterol did not form any hydrogen bond with the binding site but did form π-alkyl interaction, one of which is PHE518 residue. The previous study reported that interaction with PHE518 residue is linked with anti-inflammatory activities of compounds extracted from *E. arvense* [16–18].

On the other hand, quercetin formed the same hydrogen bond with Mefenamic Acid with several other additional hydrogen bonds (Figure 2). Despite the similarities in the interaction, Mefenamic Acid binds stronger with the COX-2 binding site than quercetin as proven as the binding energy. Weaker binding of quercetin suggesting that while the ligands have anti-inflammatory agent activity, it is weaker than Mefenamic Acid. This result is in agreement with the previous study that reported that quercetin is a weak inhibitor to Arachidonic acid biosynthesis by phospholipase A2 (PLA2) [19]. While further studies using other more advance results are needed, based on the available results and discussion above, researchers recommend 28-isofucosterol as a potential anti-inflammatory agent.

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**Figure 1.** Visualization of the interaction of ligands and COX-2 receptors.

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![Image](28-Isofucosterol-COX-2)

![Image](β Sitosterol-COX-2)

![Image](Sitosterol-COX-2)

(Van der waals) (π -alkyl) (π -sigma) (Conventional hydrogen bond)
Figure 2. 3D visualization of COX-2 complex with quercetin ((a) - (b)), COX-2 complex with 28-
isoferosterol ((c) - (d)), and hydrophilic (e) and hydrophobic (f) binding site.

4. Conclusion
We have performed an investigation on 8 compounds isolated from Ficus religiosa and predict their
inhibition on the COX-2 receptor using molecular docking calculation. Calculation results show that 28-
isoferosterol, β-sitosterol, and sitosterol lower in.

Acknowledgement
Financial support for this research by Universitas Islam Negeri Sulthan Thaha Saifuddin Jambi is
gratefully acknowledged.

References
[1] Sethi R, Gómez-Coronado N, Walker AJ, Robertson O D, Agustini B, Berk M and Dodd S 2019
Psychiatry 10 1–21
[2] Kargutkar S and Brijesh S 2018 Inflammopharmacology 26 469–477
[3] Fabiola V, Ralf T K, Gabriel B, Ermilo A V, Torre V, Martha M and Mirbella C 2016 J.
Ethnopharmacol. 190 174–182
[4] Tasneem A S, Liu B, Li B and Iqbal M 2018 Pharmacol. Res. 139 126-14
[5] Rathinavel T, Ammashi S and Shanmugam G 2017 Int. J. Adv. Interdiscip. Res. 4 6–14
[6] Martin Arias L H, Martin González A, Sanz Fadrique R and Vazquez E S 2019 J. Clin.
Pharmacol. 59 55–73
[7] Singh D, Singh B and Goel R K 2011 A review, J. Ethnopharmacol. 134 565–583
[8] Sandeep V K, Kumar A, Dimple, Tomer V and Gas Y 2018 J. Pharmacogn. Phytochem. 7 32–
37
[9] Hannan M A, Sohag A A M, Dash R, Haque M N, Mohibullah M, Oktaviani D F, Hossain M T,
Choi H J and Moon I S 2020 Phytoestrogens of marine algae: insights into the potential health benefits and molecular pharmacology Phytomedicine 153201

[10] Mamidisetti Y D, Yammada N and Kumar H 2018 Pharma Innov. 7 69–74

[11] Singh S and Jaiswal S 2014 Int. J. Eng. Res. Gen. Sci. 2 149–158

[12] Shah K, Mujwar S, Gupta J K, Shrivastava S K and Mishra P 2019 Assay Drug Dev. Technol. 17 285–291

[13] Loizou S, Lekakis I, Chrousos G P, Moutsatsou P 2010 Mol. Nutr. Food Res. 54 551–558

[14] Paniagua-Pérez R, Flores-Mondragón G, Reyes-Legorreta C, Herrera-López B, Cervantes-Hernández I, Madrigal-Santillán O, Morales-González J A, Álvarez-González I and Madrigal-Bujaidar E 2017 African J. Tradit. Complement. Altern. Med. AJTCAM. 14 123–130

[15] Gong Y, Xue B, Jiao J, Jing L and Wang X 2008 J. Neurochem. 107 779–788

[16] Ikeda A, Funakoshi E, Araki M, Ma B, Karuo Y and Tarui A 2019 Bioorg. Med. Chem. 27 1789–1794

[17] Levita J, Rositama M R, Alias N, Khalida N, Saptarini N M and Megantara S 2017 J. Appl. Pharm. Sci. 7 103–110

[18] Do Monte F H M, Dos Santos J G, Russi M, Bispo Lanziotto V M N, Moreira Leal L K A and De Andrade Cunha G M 2004 Pharmacol. Res. 49 3 239–243

[19] Dmello P, Gadhwal M K, Joshi U and Shetgiri P 2011 Int. J. Pharm. Pharm. Sci. 3 4 33–40