**In silico** anti-inflammatory activity evaluation of some bioactive compound from *Ficus religiosa* through molecular docking approach

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**Abstract.** This tree generally is used as traditional medicine for several ailments. In this research, the *In Silico* approach using molecular docking has been applied to 11 compounds from the *Ficus religiosa* to the cyclooxygenase-2 (COX-2) receptor. This study aimed to predict the ability of 11 compounds from the *Ficus religiosa* in inhibition COX-2 enzyme as a prostaglandin source. The detailed information was obtained using the molecular docking approach. Docking simulation for 11 compounds was executed through AutodockVina embedded in MGL Tools 1.5.6. The lowest binding energy of the complexes was visualized by using Discovery Studio (Biova) software. It was found that campesterol provided the lowest binding energy to COX-2, while kaempferol strongly was tied to TYR385 and SER530 of the receptor. The compounds containing anti-inflammatory effect have the lowest binding energy, and binds to the residue as native ligands. This result is indicated that the compounds from *Ficus religiosa* have potency as an anti-inflammatory agent. Still, advanced research is needed to examine more ligands from *Ficus religiosa* to isolate the best conformation.

1. **Introduction**

Inflammatory processes in the tissue are caused by wound and infection, so immune cells and cytokines released nitric oxide (NO), prostaglandin (PG), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α). However, the extrication of NO and PG is common in inflammation [1,2]. Inflammatory symptoms in the human body can develop chronic inflammation, and it causes cancer. The inflammation must be prevented from evolving [3]. The inflammation area can react with free radicals such as hydroxyl (‧OH), superoxide (O₂⁻), and peroxyl (‧OOH, ‧OOR), and develop to more
advanced ailments. Cyclooxygenases-2 (COX-2) is a stimulus enzyme in inflammation, so the activity inhibition of this enzyme will be a target for inflammation treatment [2,4]. Non-steroidal Anti-Inflammation Drugs (NSAIDs) have been known to be able to treat inflammatory diseases. This drug has been known to be successful as an anti-inflammatory agent but had failed to inhibit COX-2 [2,4]. Furthermore, long term consumption of NSAIDs has been known to caused stomach irritation, hemorrhage, kidney, bronchus, cardiovascular system, and perforation [5–8].

Currently, the research is performed to predict the compound of a drug candidate from herbal plants. It was conducted to reduce the undesirable side effects of the long term usage of synthetic drugs. The prediction of the bioactive compound can be made by using in silico approach, molecular docking. The primary purpose of this method is to predict the affinity of a drug candidate (ligand) to bind with the protein and form the most stable complex. The molecular docking method has several advantages, such as reducing cost and time in drug design. Several results of molecular docking simulation can simplify in vivo, in vitro, and pharmacy researchers in drug modeling [9,10].

The research used some bioactive compound derived Ficus religiosa. The extract of this tree has been utilized in the medicinal system to remedy several ailments. The ancient people processed the tree using the grinding technique of leave or steam bark of trees. Recently, the scientists conducted the phytochemicals test of compounds from the extract of Ficus religiosa, and they concluded that the extract contains terpenoids, flavonoids, tannins, phenols, and glycosides. The previous study of fruit Ficus religiosa extract dose 400 mgkg⁻¹ was reported to be as effective as ibuprofen dose mgkg⁻¹ [11]. Moreover, the methanolic leaf extract Ficus religiosa inhibited samples induced NO, TNF-α, IL-1β [12]. The presence of glycosides dan tannins has an activity for healing the wounds compared to the standard (ibuprofen gel) [13].

The Ficus religiosa is prominent as anti-diarrhea, anti-fungal, anti-plasmodial, anti-ulcerogenic [14], antimicrobial, antidiabetic, anticonvulsant, wound medicine, dan anti-inflammatory [15]. These abilities are due to phytochemical content such as tannins, saponins, flavonoids etc [16,17]. The previous research has shown that anti-inflammatory is obtained from methanolic extract of bark and fruit of Ficus religiosa [11,12,14,18,19]. The study of compounds derived from Ficus religiosa and COX-2 receptor, especially anti-inflammatory through molecular docking, is still minimal, so it has to conduct for the database. 5IKR is one of the protein data banks that file coordinates, and structure factors of COX-2 and it binds native ligand, mefenamic acid [20]. The studies of COX-2 inhibition on 5IKR have been developed by using bioactive compounds from plants [1,2,4–6,21,22]. Based on the explanation above, this study has performed molecular docking simulation between 11 compounds from Ficus religiosa to the COX-2 receptor (PDB ID; 5IKR) to find the anti-inflammatory effect of the best conformation. The selected compounds were collected from a previous study [23].

2. Methods
The macromolecule used in this study is chain A of Cyclooxygenase-II complex (PDB ID: 5IKR) retrieved from www.rcsb.org. The macromolecule is then prepared using Chimera 13.1. The ligands used were selected from the list of compounds found in ref [23] and retrieved from www.pubchem.ncbi.nlm.nih.gov. Docking is performed using 100 runs of the Lamarckian Genetic Algorithm with the grid box sized of 40 × 40 × 40 and spacing of 0.375 Å on Autodock suits software [5,24]. The results of docking are then analyzed using Autodock Tools suits software, and the interaction is visualized using Biovia Discovery Studio Visualizer [4]. The work details of this study to get the data are listed in Figure 1.
3. Result and Discussion

*In silico* approach commonly is the method for prediction and confirmation of drug design. This method has advantages such as inexpensive, reduce time-consuming, and minimize the isolation of inactive compounds [5,9]. The study has been done using MGL Tools based Autodock software between the selected compounds from *Ficus religiosa* and COX-2 receptor (PDB ID: 5IKR). The final result compared to the control of ligand mefenamic acid. Before the research was started, the method validation was conducted to ensure data accuracy. On the validation process, the grid box was set to 40 x 40 x 40 Å. The selected compounds were summarized from the previous study [23]. LGA was used to score of molecules. After the molecular docking simulation process yielded binding energy information, the lowest energy of complex was evaluated using Discovery Studio (Biovia). The result of molecular docking simulation between 11 compounds from *Ficus religiosa* to the COX-2 receptor is listed in Table 1.

Table 1. Analysis of inhibiting potency of *Ficus religiosa* against the COX-2 receptor with *in silico* approach.

| No. | Compounds     | Binding Energy (kcalmol⁻¹) | RMSD (Å) | Amino Acid          |
|-----|---------------|----------------------------|----------|---------------------|
| 1   | Aromadendrene | -8.04                      | 2.75     | -                   |
| 2   | Bycyclogermacrene | -6.81                 | 2.06     | -                   |
| 3   | Campesterol   | -11.83                     | 2.76     | -                   |
| 4   | Kaempferol    | -7.27                      | 1.84     | **SER530, TYR385,** |
|     |               |                            |          | **TYR355, ARG120,** |
|     |               |                            |          | **MET522**          |
| 5   | Humulene      | -7.23                      | 1.79     | -                   |
| 6   | Lanosterol    | -4.92                      | 4.09     | -                   |
| 7   | Lupeol        | -11.59                     | 2.72     | -                   |
| 8   | Myricetin     | -6.3                       | 2.35     | **TYR355, SER350,** |
|     |               |                            |          | **TYR385, MET522,** |
|     |               |                            |          | **ARG120**          |
The previous study showed the isolated from traditional medicinal plants inhibited inflammation processes on Arachidonic Acid (AA) pathway [25]. In this pathway, the bioactive compounds from the medicinal plant inhibit the performance of the COX-2 receptor when this receptor changes AA into prostaglandin, called prostaglandin biosynthesis. As we know that PG is a major mediator of inflammation process on the membrane. The selected compounds from *Ficus religiosa* are expected to replace of NSAIDs group. NSAIDs have negative effects for long usage, so usage of a drug derived from plants is an alternative way. Because the utilization of bioactive compounds from the herb can potentially reduce undesirable side effects to a few side effects. The previous study yielded that the isolated gentiopicroside from *Gentiana officinalis* H. Smith was strongly tied to the COX-2 receptor with binding energy \(-13,329\) kcalmol\(^{-1}\) [1].

Based on Table 1, No. 12 showed that the value \(-7.59\) kcalmol\(^{-1}\) and 0.58 for binding energy and RMSD of mefenamic acid, respectively. The RMSD value less than two determined that the conformation of mefenamic acid-COX-2 is deliberated as efficacious docking, and it is close to crystallographic pose [5]. Mefenamic acid as a reference inhibitor resulted in bound trough TYR385 and SER530 residues. The redocking result is compromised to the previous study that the COX-2 receptor interacts with TYR385 [20,26]. As can be seen in Table 1, aromadendrene, campesterol, kaempferol, lupeol, myricetin, and stigmasterol compounds displayed the binding energy close to and lower the reference compound (mefenamic acid). While the binding energy of bycyclogermacrene, humulene, lanosterol, myricetin, z-3-hexenol, and z-3-hexenyl acetate are lower than the reference ligand, so it indicates these compounds have not able to inhibit the COX-2 receptor.

Kaempferol strongly ties to SER530, TYR385, TYR355, ARG120, and MET522 on the active site of the receptor, and this research was similar to the prior study (Fig. 2) [26]. Kaempferol has same interaction to a residue with reference ligand (TYR385 and SER530), but the binding energy is lower than reference ligand. It indicates that kaempferol is not strong enough to inhibit the COX-2 receptor. This result is not the same as the previous study that claimed kaempferol could interact with TYR385 and SER530 on the active site of the COX-2 receptor [4]. Campesterol has the lowest binding energy, but it was not bound to active site at the COX-2 receptor. It was played on aromadendrene, lupeol, and stigmasterol with binding energy \(-8.04; -11,59; -9.83\) kcalmol\(^{-1}\), respectively. The presence of sugar groups at the molecule can cause high hydro facility. These criteria reduce bioavailability, acceleration of metabolism, shortening of half-life, and restriction of the effectiveness [1]. The 3D and 2D visualizations between campesterol, kaempferol, and mefenamic acid were displayed in Figure 2.

The binding energy of lupeol is lower than reference, ligand but it is not tied to residue on the active site of the protein. The prior study reported lupeol could inhibit formation PG on A23187-stimulated macrophages and it reduces myeloperoxidase levels, neutrophil specific marker [27]. On the other hand, lupeol derived from *Pimenta racemosa* offered an excellent anti-inflammatory effect, and the effect is as effective as indomethacin, the selective cyclooxygenase inhibitor [28]. Recently, the performance of lupeol is better than α-mangosteen at the same dose, dose where 57.14% and 38.70%, respectively [29]. Nowadays, lupeol was tested on a mouse model that has bronchial asthma and it could decrease cellularity, and eosinophil levels in the bronchoalveolar fluid [30]. The isolated

| No. | Compound               | Binding Energy | RMSD | Reference Ligand |
|-----|------------------------|----------------|------|------------------|
| 9   | Stigmasterol           | -9.83          | 2.31 | -                |
| 10  | Z-3-hexenol           | -3.53          | 3.53 | VAL523           |
| 11  | z-3-hexenyl Acetate   | -4.49          | 3.54 | TYR385           |
| 12  | Mefenamic Acid (reference ligand) | -7.59          | 0.58 | TYR385, SER530  |
lupeol from *Himatanthus suculuba* inhibited edema and abdominal constrictions with dose 100 mg kg\(^{-1}\) [31].

![Figure 2](image_url)

**Figure 2.** 3D (above) and 2D (under) interaction visualization between ligand and receptor, campesterol-COX-2 (left), kaempferol-COX-2 (middle), and mfenamic acid (right).

As can be seen in Fig. 2, the interaction of three complexes such as campesterol, kaempferol, and control to the COX-2 receptor. On the campesterol complex only gave π-sigma, π-alkyl, and Van der Waals interaction. The previous study reported that campesterol has an anti-inflammatory activity through inhibition of the NF-Kb transcription. This compound is one of the phytosterol groups that inhibit cytokines pathway, including IL-6 and TNF-α [32]. Stigmasterol has the same effect with campesterol, and the binding energy of this compound is lower than reference ligand, -9.83 kcal mol\(^{-1}\). This value indicates that the formed complex is stable in interaction with the COX-2 receptor. The previous *in vitro* study showed that stigmasterol could inhibit mediator pro-inflammatory through the NF-kappa B pathway [33]. *In vivo* and *in vitro* studies explained that stigmasterol derived from *C. aromatisans* extract has an anti-inflammatory effect. The response given by stigmasterol was inhibition of the production of NO dan cytokines [32]. Therefore, the advanced investigation about the inhibition pathway needs to be done *in silico* approach to improving the result of this study. In this study, stigmasterol does not show interaction with residue on the active site of the COX-2 receptor.

4. Conclusions
The molecular docking simulation executed by Autodock is very beneficial to predict and confirm the compound of a drug candidate from *Ficus religiosa* as an anti-inflammatory agent. The selected compounds from *Ficus religiosa* have been docked to the COX-2 receptor, and campesterol has the lowest binding energy (11.83 kcalmol-1). The smaller of binding energy at the complexes, so it provides more enormous potential as an inhibitor of the COX-2 enzyme. However, campesterol did not show interaction with the residue of the COX-2. While kaempferol displayed two interactions with the residue, it indicated a excellent inhibitory ability. The results of this study have not yielded the best conformation of 11 compounds derived from *Ficus religiosa*. The compounds providing anti-
inflammatory effect have the lowest binding energy to the receptor and it binds to the residual binding as native ligands. Therefore, further research is needed using more ligands from *Ficus religiosa* to generate the best conformation of the ligand-receptor complex.

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