

# *In Silico* Anti-Inflammatory Activity Evaluation from *Usnea misaminensis* through Molecular Docking Approach

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## Abstract

*Usnea misaminensis* is an epiphytic medicinal plant from Indonesia that has several benefits, one of which is as an anti-inflammatory. This study aims to predict the ability of three compounds from *Usnea misaminensis* to inhibit the COX-2 enzyme as a source of prostaglandins using molecular docking. Receptors obtained from RSCB with PDB ID:5IKR were then prepared on UCSF Chimera 1.16 and the ligands (usnic acid, salizinic acid, and evernic acid) were downloaded 2D structure in .pdbqt format from PubChem. Docking simulation is done via AutoDock Vina 1.1.2 embedded in AutoDockTools 1.5.7. The docking results are visualized using PyMOL 2.5.2 and Biovia Discovery Studio Visualizer. Evernic acid showed binding energy (-6.8 kcal/mol) to the COX-2 receptor which was close to the binding energy value of the control ligand. Usnic acid and salazinic acid showed interactions with the same SER530 residue as the reference ligand. Compounds containing anti-inflammatory effects have the lowest binding energy and bind to residues as reference ligands. These results indicate that the compounds from *Usnea misaminensis* have potential as anti-inflammatory agents, but further research is needed to examine the potential anti-inflammatory activities.

**Keywords:** anti-inflammatory, COX-2, ligand, molecular docking, *Usnea misaminensis*

## 1. Introduction

Indonesia has abundant natural resources including medicinal plants [1]. One of them is *Usnea misaminensis*. *Usnea misaminensis* is a native plant from Indonesia which is included in the epiphytic medicinal plant and is known locally as kayu angin [2,3]. Epiphytic plants are one of the biological resources that have not been widely studied, so their use is still limited [4]. Despite its limited use, *Usnea misaminensis* is traditionally used as a medicine for fever, dysentery, inflammation, cough, canker sores, and antihypertensives [3,5]. This plant is believed to have antifatulent, diuretic, and anti-inflammatory activities [5]. The study of *Usnea misaminensis* as an anti-inflammatory agent has not been widely studied by researchers recently.

*Usnea misaminensis* has been proven to be used for several applications, such as agents for detecting hazardous substances in polluted air, for analysis of heavy metal indicators (Cu, Fe, Zn, Pb, Mn, Cr), and for

biomonitoring of potentially toxic elements (Al, Ba, Ca, Cd, Co, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, S, Sb, Sm, Sr, Tb, Th, Ti, and Zn) [6,7]. *Usnea misaminensis* has also been reported to have potential as an anti-acne based on its antioxidant, antibacterial and lipase inhibitory activities by Batubara et al. which showed that *Usnea misaminensis* was able to inhibit the growth of acne [3]. *Usnea misaminensis* also exerted cytotoxicity against oral KB-cancer cells, NCI-H187 lung cancer cells, and MCF-7 breast cancer cells [2].

The study of *Usnea misaminensis* which has the potential as anti-inflammatory was confirmed by Nugraha in his journal which stated that the extract contained usnic acid and salizinic acid [2]. This is supported by reports that usnic acid and salicylic acid have antibacterial activity by Cansaran, et al. and Candan, et al. [8,9]. Furthermore, it was also reported that both have inhibitory activity of the inflammation regulator, microsomal prostaglandin E2 synthase-1 based on the study of Bauer et al. [10]. From

several previous studies, it appears that the potential of *Usnea misaminensis* as an anti-inflammatory needs to be done.

Along with the development of technology, many studies have been carried out to predict drug candidate compounds from medical plants. This prediction can be done using molecular docking. This method can be used to determine the potential of *Usnea misaminensis* as an anti-inflammatory. This method works by predicting the affinity of the drug candidate (ligand) to bind to the protein and form the most stable complex. This molecular docking method can reduce costs and time in the drug design process. The results of this simulation can simplify in vivo, in vitro and pharmaceutical research in drug modeling [11,12].

In this study, to determine the anti-inflammatory potential of *Usnea misaminensis*, the method that was carried out by Utami [13] was used with ligands from *Usnea misaminensis* consisting of usnic acid, salizinic acid, and evernic acid [2,14]. The receptor used is COX-2 which is a stimulus enzyme in inflammation so that inhibition of the activity of this enzyme will be the target of inflammation treatment [15,16]. One of the drugs that has been circulating as a non-steroidal anti-inflammatory drug (NSAID) is mefenamate [17]. These drugs are known to be successful as anti-inflammatory agents but fail to inhibit

COX-2 [15,16]. However, long-term consumption of NSAIDs can result in serious side effects such as myocardial infarction, gastrointestinal bleeding, and renal failure [18]. Therefore, alternative anti-inflammatory compounds are needed with lower side effects. This study is intended to provide compounds that have the potential as anti-inflammatory with COX-2 inhibitory pathways.

## 2. Materials and Method

The method used in this study uses the method on Utami [13] with modifications. This study was conducted to evaluate the anti-inflammatory properties of *Usnea misaminensis*. The receptor used was the A chain of Cyclooxygenase-II protein (COX-2, PDB ID: 5IKR) which was downloaded from the [www.rcsb.org](http://www.rcsb.org) site. The receptors were then prepared to remove non-standard residues using UCSF Chimera 1.16. The ligands used in this study were usnic acid, evernic acid, and salazinic acid according to the list of compounds based on [2,14]. The compound was obtained from [www.pubchem.ncbi.nlm.nih.gov](http://www.pubchem.ncbi.nlm.nih.gov) and downloaded its structure (2D) in .pdbqt format. AutoDockTools 1.5.7 was used to create a grid box with a size of 10 x 10 x 10 and a spacing of 1.000. Docking is done using Autodock Vina 1.1.2. The docking results are visualized using PyMOL 2.5.2 and Biovia Discovery Studio Visualizer. The stages in this research are described in Fig. 1.

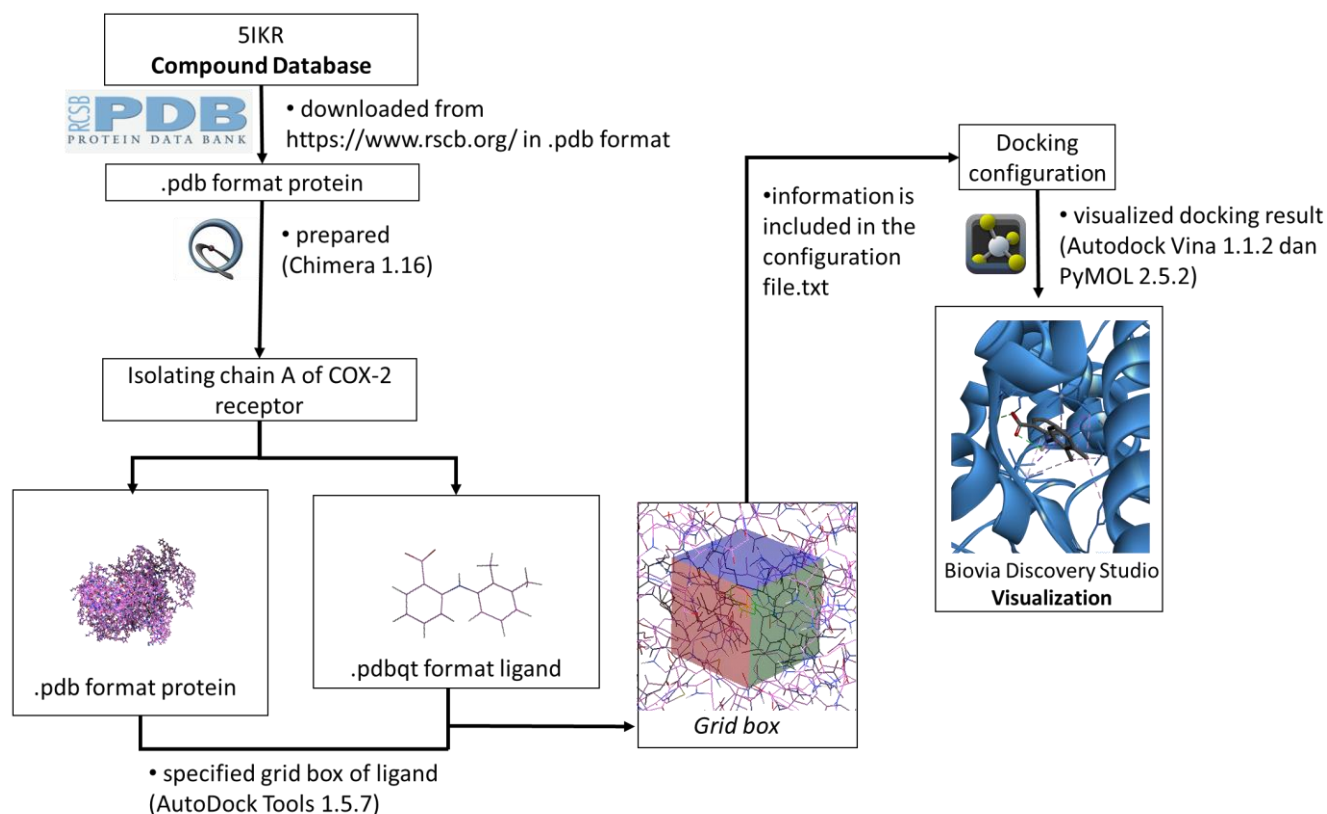


Figure 1. Flowchart of anti-inflammatory evaluation research in *Usnea misaminensis*.

### 3. Results and Discussion

In this study, we used an *in silico* approach which is commonly used in predicting drug candidates. In this paper, mainly evaluating the anti-inflammatory activity of compounds on *Usnea misaminensis* as a ligand and COX-2 (ID: 5IKR) as a receptor has been carried out using the Autodock Vina software. The results obtained were then compared with the control ligand mefenamic acid after previously validated the method using AutoDockTools to ensure the accuracy of the data obtained. Information on binding energy was obtained after the molecular docking simulation was completed. Then visualized with Biovia Discovery Studio Visualizer. The results of the simulations carried out are presented in Table 1.

**Table 1.** Analysis of the anti-inflammatory potential of *Usnea misaminensis* at COX-2 receptors using an *in silico* approach.

No.	Compound	Binding Energy (kcal/mol)	Amino Acid
1	Mefenamic Acid	-9.1	<b>TYR385, SER530</b>
2	Usnic Acid	-6.3	ARG120, <b>SER530</b>
3	Evernic Acid	-6.8	TYR355, ARG120
4	Salazinic Acid	-6.6	<b>SER530</b>

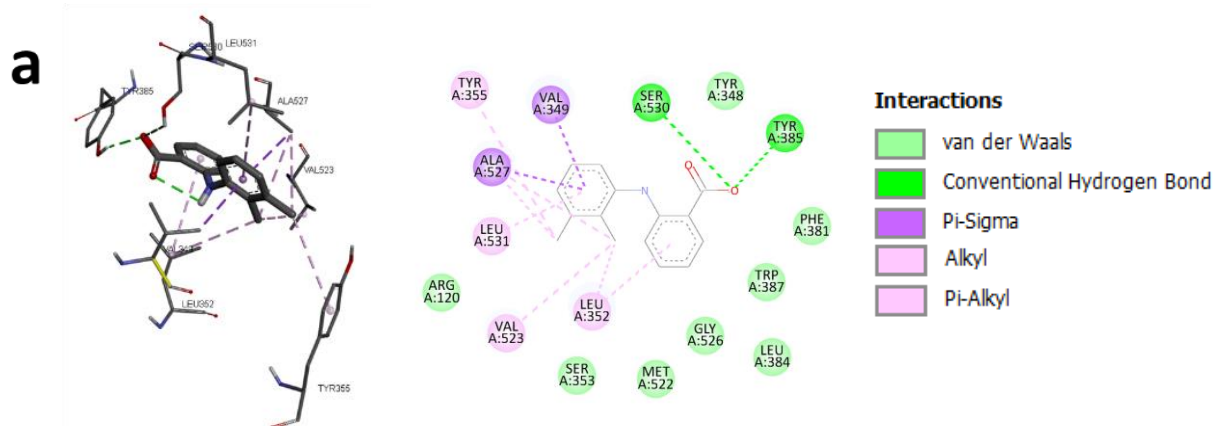
Mefenamic acid has a binding energy value of -9.1 kcal/mol and an RMSD value of 1.489 Å. An RMSD value of less than two indicates that the conformation of mefenamic acid-COX-2 is considered an efficacious docking agent, and is close to the crystallographic pose [19]. Mefenamic acid as a reference inhibitor forms bonds with residues TYR385 and SER530. Previous studies have suggested that the COX-2 receptor interacts with TYR385

[20,21]. Table 1 shows that all compounds, namely usnic acid, evernic acid, and salazinic acid, have lower binding energies than the reference ligand (mefenamic acid), indicating that all of these compounds have not been able to inhibit COX-2 receptors.

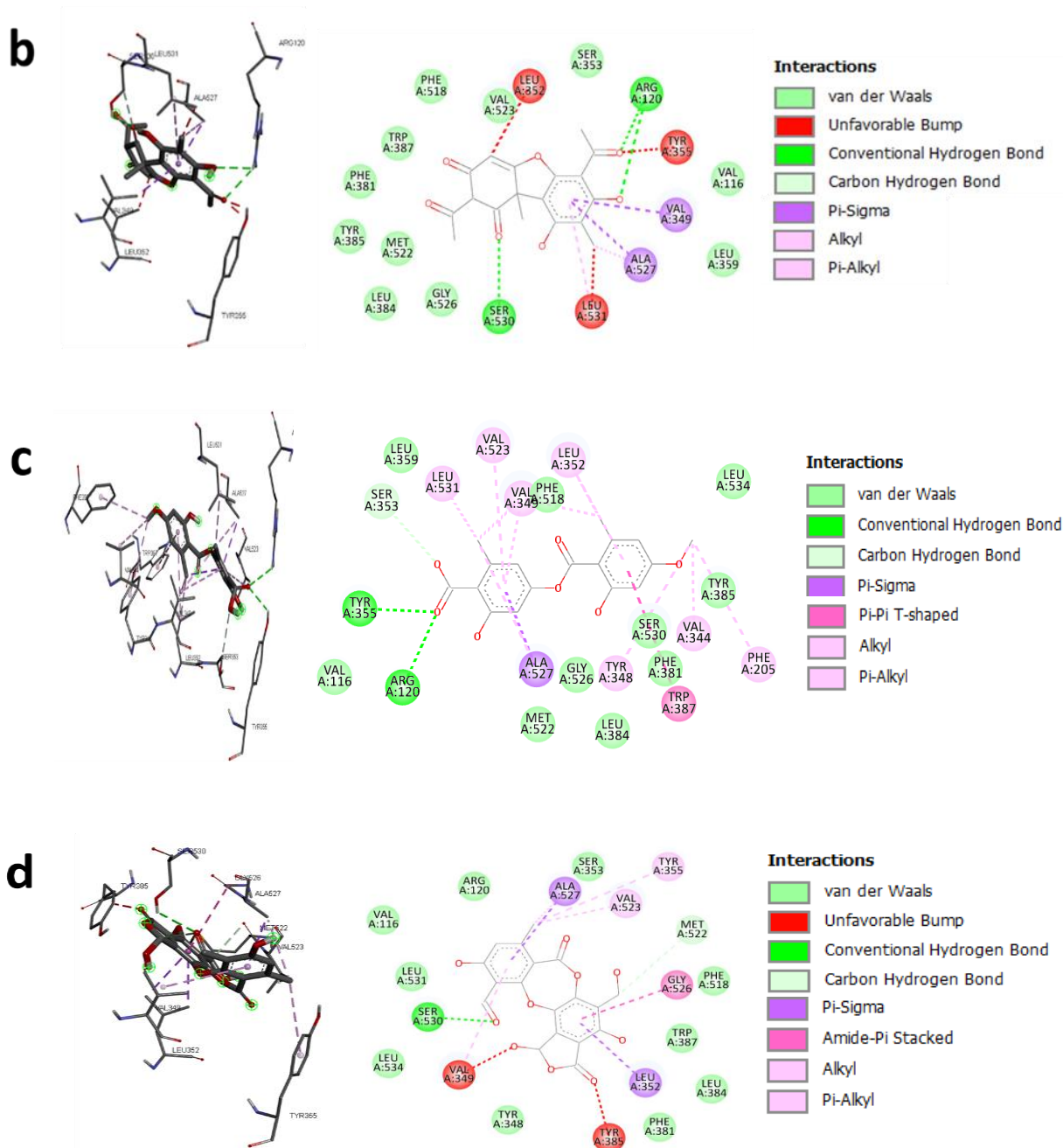
Usnic acid binds to the SER530 and ARG120 active site receptors. Usnic acid has the same interaction with the residue as the reference ligand (SER530), but the binding energy is lower than that of the reference ligand. This indicates that usnic acid is not strong enough to inhibit COX-2 receptors. However, usnic acid can be considered as a potential drug candidate for COX-2 inhibition because of its promising binding affinity [22].

Evernic acid docking results showed the best binding energy of the other two ligands in *Usnea misaminensis*, but not better than control ligands. This shows the potential of evernic acid as an inhibitor of COX-2 enzyme, although evernic acid does not have the same interaction with the reference ligand residues. These results are consistent with previous studies which stated that evernic acid can reduce MPP<sup>+</sup>-induced COX-2 levels (downstream inflammatory factor NF-κB) [22].

The results of salazinic acid docking showed that the binding energy was close to the control ligand and bound to the SER530 residue. This interaction is similar to that of the reference ligand. This shows good inhibitory ability, but not better than control ligands. In accordance with previous studies which stated that salazinic acid may have inhibitory activity against the COX-2 enzyme when using the control ligands celecoxib and rofecoxib [23]. 3D and 2D visualizations between mefenamic acid, usnic acid, salazinic acid, and evernic acid are shown in Fig. 2.



**Figure 2.** Visualization of molecular interactions between ligands and receptors (a) mefenamic acid, (b) usnic acid, (c) evernic acid, and (d) salazinic acid (continued on the next page).



**Figure 2.** Visualization of molecular interactions between ligands and receptors (a) mefenamic acid, (b) usnic acid, (c) evernic acid, and (d) salazinic acid (continued from previous page).

Table 2 shows that mefenamic acid, usnic acid, evernic acid, and salazinic acid have amino acid interactions with COX-2 receptors. While residues act as an active role in mefenamic acid are TYR385 and SER530, in usnic acid are ARG120 and SER530, in evernic acid are TYR355 and ARG120, and in salazinic acid is SER530.

In the evernic acid-COX-2 interaction, there was no unfavorable bond, indicating that evernic acid was the most stable ligand compared to the other 2 compounds. This is because unfavorable bond can damage the intermolecular forces and show the presence of intermolecular repulsion forces [24].



**Table 2.** Results of visualization of molecular interactions.

No.	Compound	Interaction
1	Mefenamic Acid-COX-2	Hydrogen bond: SER530, TYR385 Van der Waals bond: ARG120, SER353, MET522, GLY526, LEU384, TRP387, PHE381, TYR348 $\pi$ bond: ALA527, VAL349, TYR355, LEU531, VAL523, LEU352
2	Usnic Acid-COX-2	Hydrogen bond: ARG120, SER530 Van der Waals bond: SER353, VAL116, LEU359, GLY526, LEU384, MET522, TYR385, PHE381, TRP387, PHE518, VAL523 $\pi$ bond: VAL349, ALA527 Unfavorable bond: LEU352, TYR355, LEU531
3	Evernic Acid-COX-2	Hydrogen bond: TYR355, ARG120 Van der Waals bond: LEU359, PHE518, LEU534, TYR385, SER530, PHE381, LEU384, GLY526, MET522, VAL116, SER353 $\pi$ bond: LEU531, VAL523, VAL349, LEU352, PHE205, VAL344, TRP387, TYR348, ALA527
4	Salazinic Acid-COX-2	Hydrogen bond: SER530 Van der Waals bond: VAL116, ARG120, SER353, MET522, PHE518, TRP387, LEU384, PHE381, TYR348, LEU534, LEU531 $\pi$ bond: ALA527, TYR355, VAL523, GLY526, LEU352 Unfavorable bond: VAL349, TYR385

#### 4. Conclusions

Drug candidate compounds from *Usnea misaminensis* as anti-inflammatory agents can be predicted and confirmed through molecular docking simulations using AutoDock. Evernic acid from *Usnea misaminensis* showed binding energy to the COX-2 receptor which was close to the binding energy value of the control ligand with a binding energy of -6.8 kcal/mol. This shows its potential as an inhibitor of the COX-2 enzyme. However, evernic acid showed no interaction with COX-2 residues. Meanwhile, usnic acid and salazinic acid showed the same interaction with the SER530 residue as the reference ligand. This shows that both acids have poor inhibitory potential. The results obtained showed that the potency of *Usnea*

*misaminensis* as an anti-inflammatory is less good when compared to mefenamate to inhibit the COX-2 enzyme. However, these results need to be investigated further in vitro or in vivo. In addition, other secondary metabolites from *Usnea misaminensis* extract can also be studied.

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