Molecular Docking Approach of Bryophyllum Pinnatum Compounds as Atherosclerosis Therapy By Targeting Adenosine Monophosphate-Activated Protein Kinase and Inducible Nitric Oxide Synthase

Yuyun Yuniwati¹, Mokhamad Fahmi Rizki Syaban², Salsabila Ghina Anoraga², Faradilah Lukmana Sabila²

¹Department of Radiology, Saiful Anwar General Hospital-Faculty of Medicine, Brawijaya University, Malang, Indonesia
²Faculty of Medicine, Brawijaya University, Malang, Indonesia

Corresponding author: Yuyun Yuniwati, Professor, Department of Radiology, Saiful Anwar General Hospital-Faculty of Medicine, Brawijaya University, Malang, Indonesia, Jaksan Agung Suprapto No.2, Klojen, Malang, East Java, Phone, yuyun@ub.ac.id, E-mail: yuyun@ub.ac.id. ORCID ID: https://orcid.org/0000-0001-9932-2015.

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ABSTRACT
Background: Bryophyllum pinnatum is a herbal medicine from Indonesia which has an anti-inflammatory effect. Adenosine monophosphate-activated protein kinase (AMPK) and inducible nitric oxide synthase (iNOS) play a function in thickening and inflammation in atherosclerosis disease. Objective: This research aims conducted to know the potential of Bryophyllum pinnatum as a therapy for atherosclerosis by targeting AMPK and iNOS. Methods: We employed a molecular docking technique to interact active compounds of Bryohyllum pinnatum with AMPK and iNOS, which were retrieved on the protein databank. Molecular docking analysis utilizing tools such as Pyrx 9.5, Pymol, and Discovery Studio, to support the interaction between the compound and protein. Molecular Dynamic (MD) simulation also performed using CABS-FLEX 2.0 server to know the stability interaction. Results: Bryophillin B was an active compound that possesses significant binding to AMPK and iNOS. It had same binding pocket as native ligand, and Bryophyllin B has a stronger interaction with AMPK. Based on the RMSF, the interaction binding complex Bryophyllin B with AMPK and iNOS were stable. Conclusion: Bryophillin B was predicted to have potential therapy for atherosclerosis disease.

Keywords: Bryophyllum pinnatum, AMPK, iNOS, In Silico, Herbal medicine.

1. BACKGROUND
Atherosclerosis is the main cause of vascular disease worldwide, such as ischemic stroke, peripheral arterial disease, and cardiovascular disease. Cardiovascular disease grows disproportionately in middle-low countries over the world with a mortality rate of 80%, and day by 2030 predicted 23.6 million people would die (1, 2).

Obviously, this concept of atherosclerosis over quarter-century inflammation being a primordial role in atherogenesis. Based on Virchow’s theory tells that atherosclerosis is a process of thrombus formation that action occurs in blood vessels through fatty degeneration, which directly also increases the inflammatory reaction in cells. This happens continuously, which it’s impossible to regard as simply passive processes, resulting in a transformation due to inflammation (3).

The growth of atherosclerosis is linked to the vascular inflammation that is involved by iNOS and AMPK pathways. iNOS has a role as an endogenous vasoconstrictor, formation of vascular lesion, and play infiltration of inflammatory cells (4) 28.1% in non-diabetic CAD patients and 12% in controls. The T allele frequency was higher in the nondiabetic CAD group (14%. AMPK has a major role in homeostasis involving intracellular activities, activity for reuptake of glucose, and the oxidation of fatty acid (5).

According to that pathways, Bryophyllum pinnatum leaves extract consists of the active compounds that had a role in anti-inflammatory agents (6). These compounds generate anti-inflammatory products implicated in every stage of atherosclerosis, from the initiation of atheroma to destabilization. Recently study about herbal medicine was highly concerned. The research about the effect of active compounds
Table 1. Target Protein Structural Information.

<table>
<thead>
<tr>
<th>Name</th>
<th>PDB ID</th>
<th>Visualization Method</th>
<th>Resolution</th>
<th>Atom count</th>
<th>Weight (kDa)</th>
<th>Chain</th>
<th>Sequence Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK</td>
<td>3AQV</td>
<td>X-ray diffraction</td>
<td>2.08 Å</td>
<td>2301</td>
<td>31.97</td>
<td>1</td>
<td>276</td>
</tr>
<tr>
<td>iNOS</td>
<td>4NOS</td>
<td>X-ray diffraction</td>
<td>2.25 Å</td>
<td>15317</td>
<td>201.82</td>
<td>1</td>
<td>427</td>
</tr>
</tbody>
</table>

Table 2. Binding Affinity interaction.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding Affinity (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMPK</td>
</tr>
<tr>
<td>Bryophyllin A</td>
<td>-9.3</td>
</tr>
<tr>
<td>Bryophyllin B</td>
<td>-9.9</td>
</tr>
<tr>
<td>Bryotoxin A</td>
<td>-8.8</td>
</tr>
<tr>
<td>Bryotoxin B</td>
<td>-8.7</td>
</tr>
<tr>
<td>6-[(2-piperidin-1-ylmethoxy)pheno]-3-pyridin-4-ylpyrazolo[1,5-alpyrimidine</td>
<td>-9.3</td>
</tr>
<tr>
<td>2-amino-6-[1,2-dihydroxyl-propyl]-7,8-dihydro-6h-pteridin-4-one</td>
<td>-10.0</td>
</tr>
</tbody>
</table>

of *Bryophyllum pinnatum* on atherosclerosis is still not yet. Molecular docking study is the technique for screening the potential compounds as a drug for a disease. By molecular docking study, we screened the active compound of *Bryophyllum pinnatum* to be atherosclerosis therapy.

2. OBJECTIVE

The aim of the study was to investigate the potential of *Bryophyllum pinnatum* as a therapy for atherosclerosis by targeting AMPK and iNOS.

3. MATERIAL AND METHODS

Ligands and Protein Preparation

*Bryophyllum pinnatum* contains the active compounds Bryophyllin A, Bryophyllin B, and Bryotoxin A, Bryotoxin B from our previous study. The chemical compounds were downloaded from PubChem database as sdf file. Besides, protein samples for AMPK and iNOS were generated using the RCSB database (https://www.rcsb.org/). Native ligands are also obtained on the web protein data bank, which is utilized as a comparison of interactions with the active chemical *Bryophyllum pinnatum* (7).

Anti-inflammation Bioactivity Prediction

The anti-inflammation bioactivity prediction of each compound was performed using the Way2Drug PASS Online web server (http://www.pharmaexpert.ru/passonline/) with entered SMILE code for each compound. The result was Pa (Probability Activity) and Pi (Probability inhibition). Pa must be more than 0.3, whereas Pi (Probability Inhibition) must not exceed Pa.

Ligands and Protein Interactions

Molecular interactions on molecular complexes produced by docking simulations were analyzed using the Discovery Studio software version 16.1.0. This allowed us to discover the chemical bonds that had been formed. In two-dimensional structures, a wide variety of chemical linkages were shown, such as hydrogen, hydrophobic, and electrostatic interaction (11, 12). PyMol structural selection and coloring software was used to represent the difficult three-dimensional structure of the mooring simulation results, which were obtained by simulation. Sticks, cartoons, ribbons, spheres, and surfaces served as the basis for the software’s interface (13).

4. RESULTS

All samples were aligned in the laboratory using the X-ray method, and then the protein had A chains with a maximum length of 400 mer, and a minimum length of 260 mer for each target protein was sequenced. AMPK structure had obtained with the code (3aqv) and iNOS (4nos). AMPK structure had a resolution of 2.08 Å, and iNOS had a resolution of 2.25 Å, as shown in Table 1. Meanwhile, in silico protein resolution refers to the clarity of the atomic distance between amino acid residues when presented in software; the greater the value, the more detailed the molecular visualization (14). AMPK is composed of a catalytic subunit alpha and two regulatory subunits, beta and gamma (15). In humans, there are two subunit isoforms, 1 and 2, which share 90% and 61% of their amino acid sequences inside the catalytic domain and the remaining C-terminal half area, respectively. Compound C ([6-[(2-piperidin-1-ylmethoxy) phenyl]-3-pyridin-4-ylpyrazolo[1,5-alpyrimidine] is a selective inhibitor of AMPK (16). While, The crystallized iNOS oxygenase domain (iNOSox) consists of the complete catalytic domain complexed with iron protoporphyrin IX (heme), BH4, and a single structural zinc atom (17). Analyzed results from the probability effect of *Bryophyllum pinnatum* compounds.
**Molecular Docking Approach of Bryophyllum Pinnatum Compounds**

*Bryophyllum pinnatum* as an anti-inflammatory agent shown in Figure 1. Bryophyllin A had the highest inflammatory effect, following with Bryophyllin B. The higher probability value shown, the greater possibility effect given (18). If $P_a > 0.7$, the compound predicted has the same activity in the experiment. If $0.5 < P_a > 0.7$, the drug is more likely to display the activity in an experiment, but the probability is lower. If $P_a$ is less than 0.5, it is improbable that the drug will display has the same activity as the experiment (19). However, true experimental is needed to know the anti-inflammatory activity of each compound.

Bond strength was predicted using a grid box with the center coordinates and dimensions shown below. The coordinates were chosen based on the location of a novel ligand-binding pocket identified in the literature research. Coordinates and dimensions for AMPK (X: -4.767 Y: 47.767 Z: 8.615; X: 23 Y: 23, Z: 23), and iNOS (X: 0.694 Y: 96.553 Z: 19.798; X: 23, Y:23, Z:23).

**Figure 1. Anti-Inflammatory Properties active compounds of Bryophyllum pinnatum**

5. **DISCUSSION**

In this study, Bryophyllin B formed the strongest interaction with AMPK and iNOS than other compounds. Bryophyllin B had a -9.9 Kcal/mol binding affinity with AMPK and iNOS. Bryophyllin B had a stronger binding affinity than native ligand, but not stronger than iNOS native ligand. This binding inhibited AMPK activity, which is involved in the invasion and accumulation of white blood cells (WBC), hence inhibiting arterial wall thickening and vascular remodeling. Besides, Bryophyllin B bonds with iNOS, which will impede vasoconstrictor activity, vascular lesions development, and inflammatory cell infiltration. The same binding to the control ligand will cause the same biological impact, so the form of contact link between the molecules created is very important to decide the resulting action potential (20, 21).

Comparing protein dynamics requires selecting one or more features that define protein dynamics and comparing their dissimilarity. Several characteristics and dissimilarity measurements have been described to capture protein dynamics conservation. The atomic root mean square fluctuations are the most basic (RMSF) (22). The results of the molecular dynamic analysis revealed a substantial change in RMSF in the absence of Bryophyllin B binding to AMPK and iNOS. The interaction between Bryophyllin B and AMPK exhibited an RMSF of 0-2 before binding (Figure 2A), but increased to 2-4 after binding (Figure 2B). This indicates that Bryophyllin B binding to the target protein domain is still stable and has biological activity. The interaction between Bryophyllin B and iNOS is more stable than the interaction of AMPK (Figure 2C, 2D). RMSF is caused by atoms interacting with peptide and protein residues at a specified distance. When the resultant distance is smaller than 4, the interaction complex is considered to be stable (23).

As seen in Figure 3, Bryophyllin B was attached to the same binding pocket site as the control, implying that it will have the same effect. The images of the ligand-protein molecular interaction in Figures 4 illustrate the binding mechanism of Bryophyllin B at the active site of AMPK and iNOS, respectively. Bryophyllin B has 2 hydrogen bonds and 4 hydrophobic bonds. Hydrogen interactions were Asp166 and Lys107. Besides, Bryophyllin B and iNOS interaction has 2 hydrogen bonds and 5 hydro-
phobic bonds. Bryophyllin B formed molecular interaction at Gly 371, Trp372, Cys 200, Ala197, Val352, and Arg 199. The data above demonstrate that the Bryophyllin B compound in Bryophyllum Pinnatum had the potential to inhibit AMPK and iNOS. In our previously study, Bryophyllum pinnatum had anti-flamatory in Systemic Lupus Erythematosus (SLE) mice model (24). AMPK has been examined utilizing numerous substances such as Bicalin, Curcumin and Gingerol (5). However, compared with our findings, Bryophyllin B has more negative bond energy, meaning that it has a stronger bond than previous research. While, iNOS over expression in atherosclerosis can be upregulated in macrophage tissues in response to inflammatory signals. Its promote pro-inflammatory activity on cell (25, 26). So, inhibition of iNOS is key role for the target therapy for atherosclerosis disease progression. However, additional study is required to assess the usefulness and toxicity of these compounds in the therapy of atherosclerosis.

6. CONCLUSION

In Sum, Bryophyllin B is a chemical found in Bryophyllum pinnatum that have potential as therapy for atherosclerosis by suppressing AMPK and iNOS activity. Additional study is required to evaluate the effectiveness and toxicity of Bryophyllin B as an atherosclerosis disease therapy.

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- Financial support and sponsorship: Nil.

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