Brazilein from secang (*Caesalpinia sappan* L.) as an antiacne agent using in silico study

C I S Arisanti¹, N L P V Paramita¹ and N P L Laksmiani ¹

¹Departement of Pharmacy, Faculty of Mathematics and Science, Udayana University, Bali, Indonesia

E-mail: lindalaksmiani@gmail.com

**Abstract.** *Propionibacterium acnes* (P. acnes) caused the inflammatory phase of acne. The applied of longterm antibiotics to eradicated p. Acne caused resistance, organ damage, and immune hypersensitivity. Strategies and development of acne treatment should be pursued. Brazilein, the active compound of secang (*Caesalpinia sappan* L.) have been proven as an anti-acne agent. This study aimed to determined the affinity and mechanism of brazilein with protein as antiacne using molecular docking. In Chimera 1.10.1 (used for protein preparation of the proteins), Hyperchem 8 (use for optimization 3D structure of brazilein), and Autodock 4.2 programs (use for molecular docking). The binding energy value and hydrogen bonds were compared with the native ligand. The binding energy values between endoglyceramidase, hyaluronate lyase, sialidases and autolysin protein with brazilein were respectively -2.34; -6.26; -7.81; and -7.17 kcal/mol. The binding energy values between endoglyceramidase, hyaluronate lyase, sialidases and autolysin protein with native ligand were -0.59; -4.4; -7.81; -4.53 kcal/mol. That binding energy indicates that brazilein had a stronger affinity and more stable than native ligand. Brazilein had an antiacne activity caused it can bind to the proteins. The mechanism of brazilein as antiacne by inhibited the protein and inhibited the progression of acne lesions.

**1. Introduction**

Acne can generally be caused by excessive production of the oil glands, hyperkeratinization of the hair follicles, oxidative stress, and release of inflammatory mediators [1, 2]. Common bacteria that cause acne are *Propionibacterium acnes* (P. acnes) which usually are present in the skin and cause of the inflammatory phase of acne [3]. Antibiotics are acne treatments that are commonly used to treat acne such as erythromycin, tetracycline, doxycycline, and minocycline. These antibiotics are usually used for an extended period [4, 5]. Long-term use can cause resistance. About 50% of individuals have experienced antibiotic resistance both topically and orally[6, 7]. It is necessary to develop antiacne drugs by using natural ingredients so that they can be used as a substitute for antibiotics to prevent and overcome the resistance of P. acne bacteria.

Secang (*Caesalpinia sappan* L.) has a MIC value of 0.13 mg/mL and MBC of 0.25 mg/mL against P. acnes. Brazilein and brazilin are the main compounds in secang, and brazilin is known to have activity as antioxidants and inhibitors of lipase enzymes in bacteria [8]. Brazilein as the other primary active compound in secang have been not yet investigated as an anti-acne agent, and the molecular mechanism of brazilein play a role in progression acne lesions have been not reported. *Propionibacterium acnes* is an organism that generally contributes to the occurrence of acne [9]. P. acnes is a gram-positive bacteria and is anaerobic with oxygen tolerance. P.acnes bacteria is a healthy
bacterial flora on the skin. The role of P. acnes bacteria in the formation of acne is to produce lipases which break down triglycerides into free fatty acids, causing inflammation. P. acnes bacteria proliferate and aggravate inflammation by stimulating the production of proinflammatory cytokines [10]. The lipoglycan-based cell envelope and their extracellular secreted lipase, particularly triacylglycerol lipase, encoded by the gehA gene assists in the adherence and the colonization of the bacterium to the sebaceous follicle. The other product which aids in the acne process by destroying the host tissue includes porphyrins, hyaluronate lyase, endoglycoceramidase, sialidases/neuraminidase, cardiolipin synthetase, and cal- calcineurin like phosphodiesterase [11, 12]. Based on this, it is necessary to evaluate the mechanism and affinity of brazilein with endoglycoceramidase, hyaluronate lyase, sialidases and autolysin protein as antiacne using in silico molecular docking. The molecular docking aims to achieve the optimized conformation and relative orientation for both the protein and ligand. Therefore, we want to know about the affinity and mechanism between brazilein with protein that plays a role in the progression of acne lesion through in silico as antiacne.

2. Method
Protein Data Bank (http://www.rcsb.org) provides The three dimensional (3D) of endoglycoceramidase (5J14); hyaluronate lyase (1F9G); sialidases (1EUS) dan autolysin (4P17). The 3D structure of brazilein was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). Protein preparation begins with choosing the protein structure in the active form that binds to a native ligand of the protein target. The first step in protein preparation is to remove the water molecules (H2O) in the target protein. Then the native ligand on target protein is eliminated by Chimera 1.10.1 program which aims to provide space (pocket/cavity) that the coordinates of the pocket and site center bindings as docking material can be known. Separate native ligands are used for the validation method process. Validation of the molecular docking method is done by docking the target protein and the native ligand again. The docking method was valid if the Root Mean Square Distances (RMSD) value \( \leq 3.0 \) Å. Then continue with docking the brazilein with the target proteins. The last procedure of this method is data analysis includes the amount of binding energy and the type of hydrogen bond formed.
3. Result and discussion

Endoglycoceramidas (5J14); hyaluronate lyase (1F9G); sialidases (1EUS) and autolysin (4P17) were downloaded from http://www.rcsb.org/pdb/home/home.do. Preparation of target proteins aimed to separate protein from the native ligand. Therefore, it is available a pocket/cavity during docking progress with brazilein.

**Figure 1.** Target Protein Structure without Native Ligand. Endoglycoceramidas (a); hyaluronate lyase (b); sialidases (c); autolysin (d)

**Figure 2.** SIA native ligand (e); ASC native ligand (f); DAN native ligand (g); AMU native ligand (h)
The protein preparation is performed by removing water molecules (H2O), so the only one that interacts is a compound test with amino acids. Those preparations were done by using Chimera 1.10.1 program that obtained protein (without native ligand) and each native ligand.

The optimization of 3D structure brazilein was done by using semi-empirical AM1 on Hyperchem 8. The first step was by calculating a single point that used to determine the total molecular energy of the structure. The energy obtained at this single point calculation is -5471.48 kcal/mol. Moreover, to employ the energy minimization algorithms that locate the stable structure was using geometric optimization calculation. The energy obtained in here is -5509.50 kcal/mol.

![Figure 3. The 3D Structure Brazilein after Single Point Calculation](image1.png)

![Figure 4. The 3D Structure Brazilein after Geometry Optimization Calculation](image2.png)

Molecular docking was performed to elucidate the molecular mechanism underlying brazilein inhibits target protein by seeing the drug-receptor binding energy. Score docking shows the bond strength between the active compound and the target protein. Lower score docking shows a stronger and more stable bond. When the bond grows stronger, the affinity between the compounds and the target protein increases. The increased of its affinity will be caused higher it’s biological activity [13].

Validation method aims to prove the parameters of the method are appropriate to use for the requirement where it’s used. The RMSD value is a parameter used to indicate that the molecular docking method is valid. RMSD value is a measurement media to compare between two poses an atomic position; those are the experimental and the predicted structure. Smaller the RMSD value that indicates the predicted ligand poses better as it approaches the native conformation [14] [15]. Validation result from target protein showed molecular docking protocol for that protein could be accepted with RMSD value < 3 Å (Table 1) and molecular docking brazilein to target protein could be continued.

**Table 1.** Redocking results between Target Proteins and Their Native Ligand

<table>
<thead>
<tr>
<th>Protein</th>
<th>RSMD (Å)</th>
<th>Binding Energy (kcal/mol)</th>
<th>Hydrogen Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>endoglyceramidase</td>
<td>2.71</td>
<td>-0.65</td>
<td>TYR302; GAL502</td>
</tr>
<tr>
<td>hyaluronate lyase</td>
<td>2.75</td>
<td>-4.4</td>
<td>ARG466</td>
</tr>
<tr>
<td>sialidases</td>
<td>0.57</td>
<td>-7.81</td>
<td>ARG68; ARG87; ARG276; ARG342</td>
</tr>
<tr>
<td>autolysin</td>
<td>1.83</td>
<td>-4.53</td>
<td>HIS22</td>
</tr>
</tbody>
</table>

Based on the results of re-docking in table 1 show, the value of RMSD is below 3 Å. Those results that indicate the method are valid. Binding energy values obtained from the docking of target proteins...
with their native ligand were compared to determine the brazilein potential as an anti-acne agent by inhibiting mechanism of that all target proteins.

In silico molecular docking between brazilein and target, proteins are performed by using the Autodock 4.2 program. The docking result showed in Table 2. Conformation with the lowest binding energy value was selected from the structure which has the most stable formation. The potential of brazilein as an antiacne which has an inhibition mechanism of endoglyceramidase; hyaluronate lyase; sialidases dan autolysin can be determined by comparing the value of binding energy with native. Based on the comparison binding energy value between the native ligand and brazilein with target proteins in Table 2, it shows that brazilein has lower binding energy value than all their native ligand except in sialidases protein. However, in the binding energy value obtained in here, it got the negative value that indicates the formation of a bond between sialidases and brazilein.

**Table 2.** Docking results between target protein with native ligand and brazilein

<table>
<thead>
<tr>
<th>No.</th>
<th>Target Protein</th>
<th>Ligand</th>
<th>Binding Energy (kcal/mol)</th>
<th>Hydrogen Bond</th>
<th>(Ligand-Protein) Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Endoglyceramidase</td>
<td>Native ligand</td>
<td>-0.65</td>
<td>TYR302 GAL502</td>
<td>O1B-HH O10,N5-H2</td>
</tr>
<tr>
<td></td>
<td>(5J14)</td>
<td>Brazilein</td>
<td>-2.43</td>
<td>GAL502</td>
<td>H-O6</td>
</tr>
<tr>
<td>2</td>
<td>Hyaluronate Lyase</td>
<td>Native ligand</td>
<td>-4.4</td>
<td>ARG466 ARG466 ASN349 ARG462 TRP292</td>
<td>O6-HH22 O5-HH12 O2-HD21 O1-HH21 O3-HE1</td>
</tr>
<tr>
<td></td>
<td>(1F9G)</td>
<td>Brazilein</td>
<td>-6.26</td>
<td>ASP293 ARG293 ASP293</td>
<td>H-OD2 H-OD2</td>
</tr>
<tr>
<td>3</td>
<td>Sialidases (1EUS)</td>
<td>Native ligand</td>
<td>-7.81</td>
<td>ARG68 ARG342 ARG276 ARG68 ARG342 ARG87</td>
<td>O1A-HH21 O1A-HH12 O1B-HH21 O1A-HH11 O1B-HH22 O4-HH22</td>
</tr>
<tr>
<td></td>
<td>Brazilein</td>
<td></td>
<td>-7.19</td>
<td>ALA93 ARG342 ARG68 ASP131</td>
<td>O-HN O-HH12 O-HH11 H-OD1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Autolysin (4P17)</td>
<td>Native ligand</td>
<td>-4.53</td>
<td>GLY162 GLY164 NAG301</td>
<td>H-O H-O N-O</td>
</tr>
<tr>
<td></td>
<td>Brazilein</td>
<td></td>
<td>-7.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The results that occur in docking of brazilein compound against target protein are binding energy and affinity. Based on table 2, brazilein has lower binding energy value than native ligand. This outcome indicates that brazilein has a stronger affinity and more stable bond than their native ligand. The interaction between proteins and ligands are reported in the form of hydrogen bonds. The hydrogen bonds showed Figure 5. Hydrogen bonds are electronegativity that can affect the bond strength of a compound. The hydrogen bond is formed in the same active side between brazilein and native ligand in among target protein. Those indicate that brazilein has potential as an antiacne by inhibiting the expression of endoglycoceramidase; hyaluronate lyase; sialidases dan autolysin with a stronger affinity. Higher its affinity will be caused higher it's biological activity.

4. Conclusions
The in silico assay with molecular docking showed that brazilein is potential as antiacne through the reducing progression acne lesion mechanism by inhibiting the activity of endoglycoceramidase; hyaluronate lyase; sialidases dan autolysin protein. The molecular docking demonstrates the high-affinity interaction between brazilein and that protein.

Acknowledgment
This research was supported by DITJEN DIKTI and Departement of Pharmacy, Mathematics and Science Faculty, Udayana University.
References


