Conference Paper

Activity of Secondary Metabolites of Momordica Charantia as a DNA Gyrase Inhibitor of Helicobacter Pylori: Homology Modelling and Molecular Docking

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Abstract.

Helicobacter pylori can induce gastritis, ulcers, and gastric cancer. *H. pylori* is also responsible for causing oropharynx, endocrine, respiratory, central nervous system, eye, and reproductive system diseases. Treatment of *H. pylori* includes relieving gastritis or pain but this is not specific for *H. pylori*. Therefore, it is necessary to develop drugs of new therapeutic molecules to treat *H. pylori*. The purpose of this study was to determine which metabolite compounds from the *Momordica charantia* plant could provide activity as a DNA gyrase inhibitor. The target protein used in this study was prepared using the homology modeling method with the SWISS-MODEL web tools. Secondary metabolite compounds of *M. charantia* were processed using SwissADME web tools to find predictions for their pharmacokinetic profiles. The secondary metabolite compounds used for molecular docking using autodock 4.2 were compounds in the BOILED-Egg method range. From the homology modeling results, the quaternary structure quality estimate was 0.57 and the global mean quality estimate was 0.52. From the BOILED-Egg, six compounds were predicted to have good bioavailability. The molecular docking found that diosgenin had the lowest binding free energy (-5.35 kcal/mol) and inhibition constant (119.52 uM), so it was predicted that diosgenin could be used as an inhibitor of DNA gyrase in *H. pylori*.

Keywords: *Helicobacter pylori*, DNA gyrase, *Momordica charantia*, homology modelling, molecular docking

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1. Introduction

Helicobacter pylori is a gram-negative, microaerophilic, spiral-shaped, and flagellated bacterium that presents a large portion of the world's population typically found in the human stomach [1, 2]. *H. pylori* is an exceptionally effective human pathogen that is ordinarily transmitted orally inside families during youth and can persevere for quite a long time in its favored specialty, the gastric mucosa, regardless of setting off enthusiastic inborn and adaptive immune response [3]. *H. pylori* infection is highly typical which prevalence of the disease differs by geographic region, age, nationality,

and financial status; truth be told, the prevalence is higher in a developing country and those with poor financial conditions [1]. Africa had the most prevalence of *H. pylori* infection (70.1%), though Oceania had the lowest (24.4%). Nigeria had the most (87.7%) of individual countries, while Switzerland had the lowest (18.9%). In 2015, there were around 4.4 billion people with *H. pylori* infection worldwide [4].

H. pylori infection causes typically gastric illnesses, however from the start of 1994, a few researchers have detailed a relationship of *H. pylori* infection with other systemic manifestations outside the stomach include various extragastric appearances, for example, neurological, dermatological, hematologic, visual, cardiovascular, metabolic, hypersensitive, and hepatobiliary diseases [1]. A few investigations have focused on understanding this bacterium's local and systemic impacts to see how *H. pylori* can create such assorted outcomes in the human host. One of the main mechanisms by which *H. pylori* harms the host is inciting local and systemic inflammation. *H. pylori* stand apart from other gram-negative bacterial pathogens in its capacity to endure and establish chronic infection [3]. The treatment of *H. pylori* is complicated, requiring at least two different antibiotics agents and gastric acid suppression for effective *H. pylori* eradication [5].

Indonesia is a country with high flora diversity and ranks seventh among all countries in the world. It is estimated that 25% of flora species are found in Indonesia, 40% are endemic flora. Unfortunately, the potential genetic resources contained in it are not fully known. Only a tiny number of floral species have been known and developed for commercial use [6].

Bitter melon fruit (*Momordica charantia*), known as pare in Indonesia, is empirically used to treat gastric diseases [7]. Previous studies have stated that polysaccharides from pare have a pharmacotherapy effect on gastritis [8, 9]. However, there is not much scientific evidence that mentions the mechanism of action. Therefore, the authors will conduct preliminary in silico research on secondary metabolites contained in *M. charantia*, which can act as DNA Gyrase inhibitors in *H. pylori*.

2. Research Methods

2.1. Secondary Metabolites of M. charantia

In this study, the ligands utilized were secondary metabolites of M. charantia, which were acquired through a search on Dr. Duke's Phytochemical and Ethnobotanical Databases

[10] and some research [11, 12]. Furthermore, the three-dimensional ligand structure was formed and optimized using Avogadro [13].

2.2. Pharmacocinetics Profile Prediction

Each secondary metabolite compound was processed using SwissADME web tools [14, 15] to find pharmacokinetic profile predictions. The secondary metabolite compounds that will be used in Molecular Docking are compounds that fall within the range of the BOILED-Egg method [15] at SwissADME.

2.3. Protein Target Preparation

The target protein used in this study was prepared using the Homology Modeling method with the SWISS-MODEL web tool [16]. The target protein sequence was obtained in GenBank [17] with the code AAA74376.1 and then processed automatically by SWISS-MODEL to get the target protein. The structure of the target protein was compared with the structure of the DNA gyrase subunit A of Streptococcus pneumoniae (PDB code 4Z2c) to make the molecular structure of the target protein.

2.4. Molecular Docking

Molecular docking of secondary metabolites was carried out using AutoDock [18] and the help of the PyRx [19]. Docking is carried out at the active point of the target protein obtained from Uniprot [20], which is around the amino acid Tyr126 with the center point of the XYZ coordinates 107.3142; -2.6120; 311.7095 with an area of 50 and spacing of 0.375. Docking is done using the Lamarckian GA algorithm, and the docking protocol number of GA runs 100, the number of individuals in population 150, the maximum number of energy evaluations 2500000, rate of gene mutation 0.02, and rate of crossover 0.8.

2.5. Data Analysis

The results of the docking used are the lowest energy estimates in the largest cluster. The results of docking that have an estimated value of inhibition constant (eKi) below 1 mM will be further analyzed of the interaction by Discovery Studio Visualizer [21]. The results of the prediction of the pharmacokinetic profile using SwissADME obtained data

as shown in Figure 1 and Table 1. The prediction results obtained eight compounds with good bioavailability because they enter the egg white part of BOILED-Egg [15].

3. Results and Discussions

3.1. Pharmacokinetic Profile Predictions

The results of the prediction of the pharmacokinetic profile using SwissADME obtained data as shown in **Figure 1**. The prediction results obtained eight compounds with good bioavailability because they enter the yolk and white areas of boiled egg. The BOILED-Egg model delivers a rapid, intuitive, easily reproducible yet statistically unprecedented robust method to predict the passive gastrointestinal absorption and brain access of small molecules useful for drug discovery and development [15].

Figure 1: Pharmacokinetic profile prediction of secondary metabolite of *M. charantia* using the BOILEDD-Egg method on SwissADME..

TABLE 1: Secondary metabolite of *M. charantia* that have good oral bioavailability predictions.

No	Compound Name
1	5-hidroxytriptamine
2	(-)-Menthol
3	α-eleostearic acid
4	β-alanine
5	Asparagine
6	Capric acid
7	Citrulline
8	Diosgenin

3.2. Protein Target Preparation

The results of homology modeling using SWISS-MODEL can be seen in **Figure 2**. To assess the quality of the results from homology modeling, we can use the Local quality estimate parameter, which is the expectation of the similarity of the model shape compared to the original structure. The x-axis represents the number of amino acid residues. At the same time, the y is a prediction of the similarity of shape to the target. The LQE value ranges from 0-1. The higher the value, the higher the prediction accuracy of the homology modeling form [22]. As seen **in Figure 3**, the model has a relatively high level of prediction accuracy.
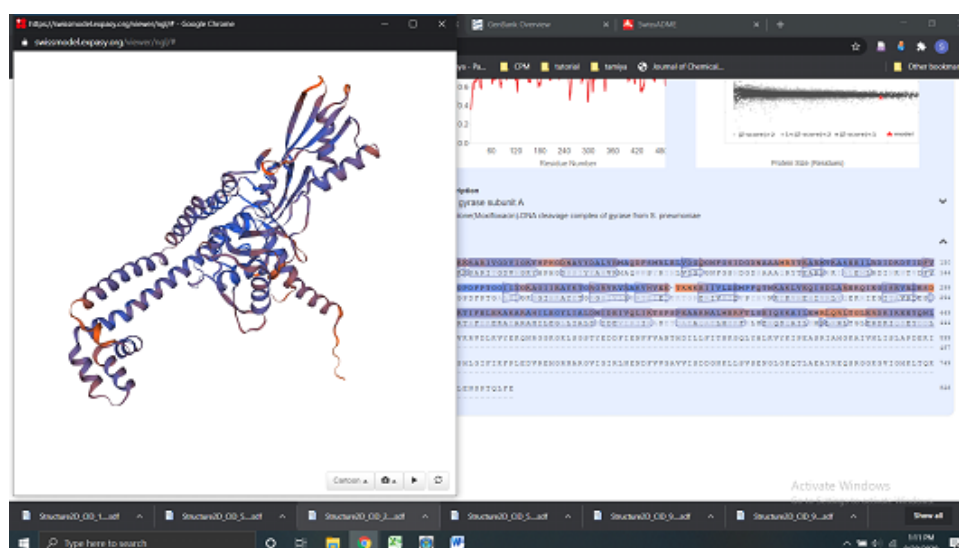

Figure 2: 3D model of *H. pylori* DNA gyrase results from homology modeling with SWISS-MODEL.

3.3. Molecular Docking

Molecular docking was carried out using six secondary metabolites that met the criteria for the BOILEDD-Egg method and were not fatty acid compounds. These compounds are excluded in the docking compound because the length of the carbon chain of the compound will make the docking accuracy decrease. From docking results (**Table 2**) can be said that there is one compound that can potentially be used as a DNA gyrase inhibitor in H. pylori, namely diosgenin. The compound has estimated free energy of binding -5.35 kcal/mol and an estimated inhibition constant (eKi) of 119.52 μM. Other compounds have eKi greater than 1 mM, so that it is predicted to be less potential if used as a candidate for DNA gyrase inhibitor.
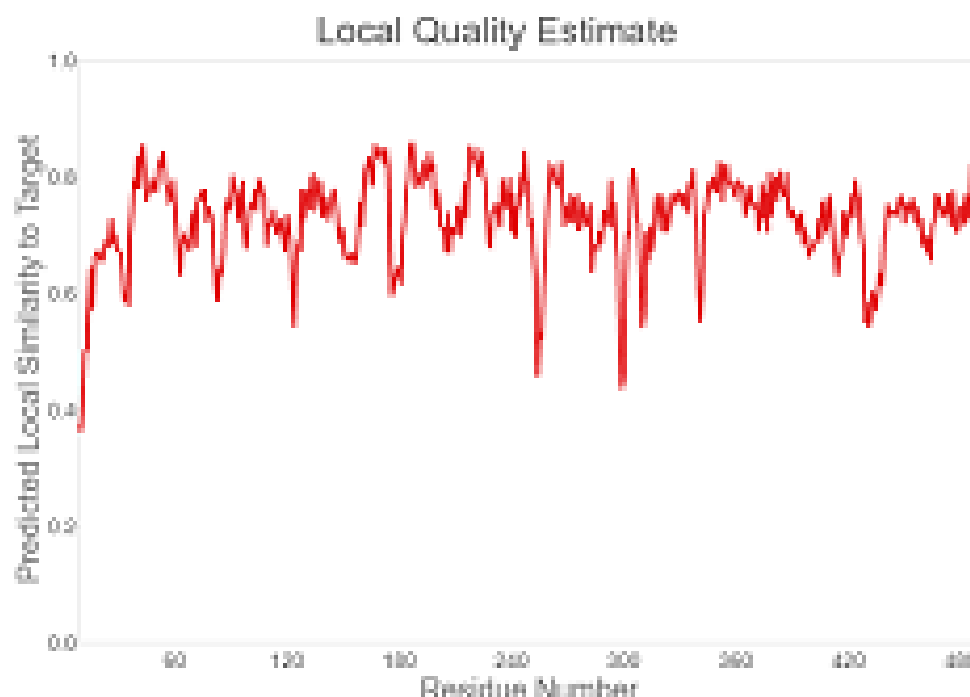
Figure 3: Local quality estimate of *H. pylori* DNA gyrase results from homology modeling with SWISS-MODEL.

Apart from estimating free energy of binding and eKi, diosgenin also has high accuracy because it is only docked in one place (cluster), and the average binding energy is the same as the smallest energy, different from other compounds docked in many areas and positions. The average energy is higher than the smallest energy, So the accuracy for diosgenin is very high.

TABLE 2: Secondary metabolite of *M. charantia* docking results on *H. Pylory* DNA Gyrase.

No	Compound Name	Estrimated Free Energy of Binding (Kcal/mol)	Mean Bind- ing Energy	Number of Cluster	Number in Cluster	Estimated Inhibition Constant
1	5-hidroxytriptamine	-3.30	-3.23	14	30	3.80 mM
2	(-)-Menthol	-3.53	-3.43	4	96	2.59 mM
3	β-alanine	-3.38	-2.27	4	79	3.34 mM
4	Asparagine	-4.08	-3.17	15	43	1.02 mM
5	Citrulline	-2.27	-1.60	20	17	3.44 mM
6	Diosgenin	-5.35	-5.35	1	100	119.52 μM

3.4. Prediction of Interaction between Diosgenin and Target Protein

The interaction between diosgenin and the target protein can be seen in **Figure 4** and **Figure 5**. Diosgenin appears to interact with the active site of the target protein residue, Tyr126. In addition, diosgenin also interacts with several other amino acid residues, namely ASN 112 and ALA 122. All of these interactions are hydrophobic interactions, and no hydrogen bonds are found in this interaction.
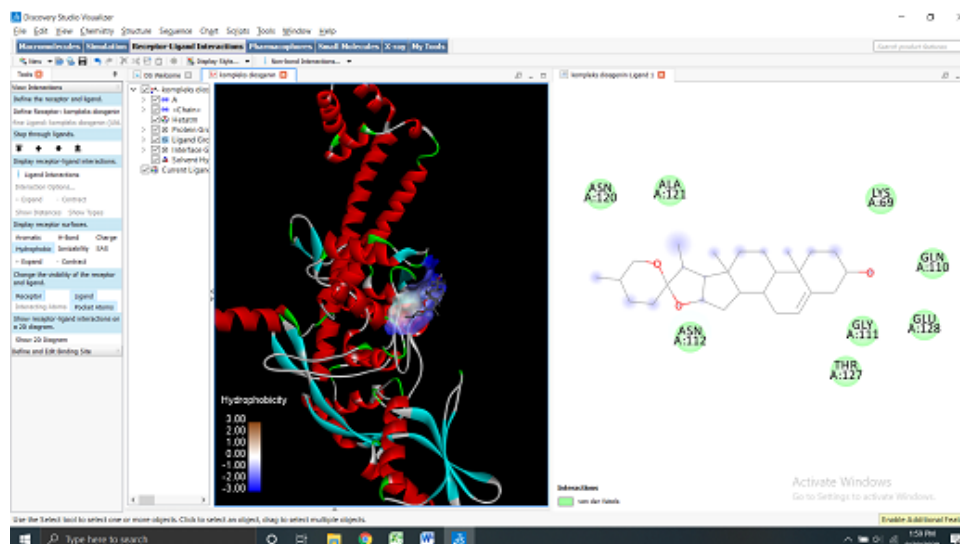

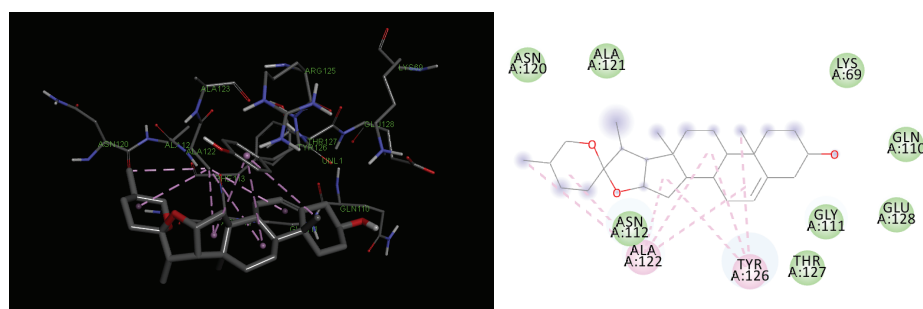**Figure** 4: 3D model of diosgenin and *H. pylori* DNA gyrase.

Figure 5: Interaction between diosgenin and *H. pylori* DNA gyrase. 3D (left), 2D (right).

4. Conclusion

Diosgenin, a secondary metabolite compound found in Momordica charantia, was predicted to have DNA gyrase inhibitor activity at h. pylori with an estimated Ki of 119.52 M and interacts directly with the protein active site residue, Tyr126.

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